

## THE EVALUATION OF TURKISH AUTUMN FLOWERING *COLCHICUM* SPECIES

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### SUMMARY

65 *Colchicum* species grow all over the World and 25 of them grow in Turkey.

In this work, Turkish *Colchicum* species with respect to the tropolone alkaloids that have gained importance in pharmaceutical industry have been evaluated. First of all, the preparative TLC separation and spectrophotometric determination of colchicine and demecolcine were accomplished. Species with low alkaloid content and with small seeds and corms which have not economic value were eliminated and 10 *Colchicum* species were investigated with respect to their total tropolonic alkaloid content using a direct spectrophotometric method. HPLC was also used to quantitate the amount of colchicine, colchicoside and demecolcine. The results obtained were compared with those of *C.autumnale* cited in the Pharmacopeiae.

As a result of our investigations, alkaloid content of *C.baytopiorum*, *bivonae*, *bornmuelleri*, *cilicicum*, *kotschyi* and *speciosum* species were found as rich as or even richer than *C.autumnale*.

### ÖZET

Dünya'da 65, Türkiye'de ise 25 *Colchicum* türü yetişmektedir.

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Türkiye'de yetişen türlerin tedavi alanında önem kazanmış tropolon alkaloidleri yönünden değerlendirilmesini amaçlayan çalışmalarda, öncelikle *Colchicum* türlerinin tohum ve yumrularında, colchicine ve demecolcine preparatif ince tabaka kromatografisi ile ayrılmış ve sonra spektrofotometrik yöntemle miktar tayini yapılmıştır. Bu yöntemle, düşük alkaloid içerdiği saptanan küçük tohum ve yumrulu türler dışında, ekonomik değeri olabileceği kabul edilen 10 *Colchicum* türü, direkt spektrofotometrik yöntemle total tropolonik alkaloidleri ve sonra da yüksek basınçlı sıvı kromatografisi yöntemiyle colchicoside, colchicine ve demecolcine miktarı bakımından, farmakopelerde kayıtlı *C.autumnale* türü ile karşılaştırılarak araştırılmış ve türler arasındaki farklar açıklanmaya çalışılmıştır.

Araştırma sonuçlarımıza göre, total alkaloid miktarı bakımından; *C.baytopiorum*, *bivonae*, *bornmuelleri*, *cilicicum*, *kotschyi* ve *speciosum* türleri, *C.autumnale* türüne eşdeğer ya da daha fazla miktarda alkaloid içermektedir.

**Key words:** *Colchicum*, Colchicine, Colchicoside, Demecolcine, Tropolon Alkaloids.

## INTRODUCTION

There are 65 *Colchicum* species in the World (1) and 25 of them grow in Turkey (2).

After the Second World War, a decrease in the amount of the drugs obtained from natural plant sources was observed because of the high cost of labour in Europe. For example, the amount of the *Colchicum* seeds obtained from *Colchicum autumnale* L. was insufficient and could not cover the demand of the alkaloid industry. Consequently, the seeds obtained from the *Colchicum* species grown in the Northeast regions of Turkey (especially *C. speciosum* Steven) were exported to foreign countries and the exported amount from Trabzon harbour, reached to approximately 45 tons in 1991 where as *Colchicum* seed was not included in the list of exported drugs in 1951.

The importance of the *Colchicum* seeds and corms for Turkey led us to work on the alkaloids of Turkish *Colchicum* and *Merendera* species(3-9).

## RESULTS AND DISCUSSION

In our studies (3) aiming the evaluation of the species grown in Turkey, with regard to the medically important tropolone alkaloids Colchicine and Demecolcine, first the seeds and the corms of the *Colchicum* species were examined by chromatographic and spectroscopic methods (Table 1). 10 of these *Colchicum* species expected to have financial value due to the rich alkaloid content (Colchicine, Colchicoside and Demecolcine) detected by the above methods were examined by HPLC and compared with *C. autumnale* recorded in the pharmacopeiae (Table 3,4). Additionally the tropolonic alkaloid quantitation was achieved by the direct spectrophotometric method and the differences between the species were determined (4).

Table 1. Spectrophotometric Determination of Colchicine and Demecolcine in *Colchicum* Species

SPECIES	ISTE	COLCHICINE (%)		DEMECOLCINE (%)	
		Corms	Seeds	Corms	Seeds
1. <i>autumnale</i>	35952	0.105	0.429		
2. <i>balansae</i>	43571	0.043	0.025		
3. <i>baytopiorum</i>	41903	0.020	0.068	K	0.469
4. <i>bivonae</i>	33849	0.058	0.089	-	0.166
5. <i>boissieri</i>	44275	0.052	Seeds not	0.031	
6. <i>bornmuelleri</i>	36184	0.138	avaible	0.118	
7. <i>chalcedonicum</i>	45852	0.043	0.065	-	0.007
8. <i>cilicicum</i>	33921	0.170	0.039	0.020	0.062
9. <i>kotschyi</i>	41498	0.111	0.113	0.014	0.151
10. <i>lingulatum</i>	44249	0.018	0.004	-	0.029
11. <i>macrophyllum</i>	36231	0.123	0.016	-	0.092
12. <i>micranthum</i>	45851	0.009	0.097	0.001	0.003
13. <i>speciosum</i>	41487	0.109	0.214	0.043	0.104
14. <i>stevenii</i>	36548	0.006	(Whole plant)	0.002	
15. <i>troodii</i>	36692	0.016	0.013	-	0.017
16. <i>turcicum</i>	42488	0.007	0.077	0.002	0.075
17. <i>umbrosum</i>	42420	0.098	0.182	-	0.087
18. <i>variegatum</i>	38989	0.032	0.015	-	0.036

Table 2. Spectrophotometric Determination of Total Alkaloids in *Colchicum* Species

SPECIES	CORMS(%)	SEEDS(%)
1. <i>autumnale</i>	1.036	1.117
2. <i>balansae</i>	0.257	0.536
3. <i>baytopiorum</i>	0.880	1.181
4. <i>bivonae</i>	0.500	1.196
5. <i>bornmuelleri</i>		
June	0.914	1.044
August	0.964	
6. <i>cilicicum</i>		
June	1.834	0.666
July	1.371	
October	0.464	
7. <i>kotschy</i>	1.014	0.939
8. <i>macrophyllum</i>	0.707	0.586
9. <i>speciosum</i>	0.782	0.797
10. <i>umbrosum</i>	0.319	0.785

Table 3. HPLC Determination of Colchicine, Colchicoside and Demecolcine in *Colchicum* Corms

SPECIES	ISTE	COLCHICINE (%)	COLCHICOSIDE (%)	DEMECOLCINE (%)
1. <i>autumnale</i>	35952	0.235	0.122	0.038
2. <i>balansae</i>	52487	0.090	0.054	-
3. <i>baytopiorum</i>	48853	0.157	0.196	0.168
4. <i>bivonae</i>	54491	0.131	0.075	-
5. <i>bornmuelleri</i>				
June	49687	0.248	0.041	0.285
August	49686	0.168	0.194	0.260
6. <i>cilicicum</i>				
July	31318	0.307	0.400	0.162
October	33921	0.354	0.200	0.205
7. <i>kotschy</i>	45336	0.223	0.108	0.211
8. <i>macrophyllum</i>	44248	0.206	0.073	-
9. <i>speciosum</i>	41487	0.227	0.113	0.194
10. <i>umbrosum</i>	49688	0.124	0.133	-

**Table 4.** Determination of Colchicine, Colchicoside and Demecolcine in *Colchicum* Seeds

SPECIES	ISTE	COLCHICINE (%)	COLCHICOSIDE (%)	DEMECOLCINE (%)
1. <i>autumnale</i>		0.398	0.571	0.025
2. <i>balansae</i>	43571	0.052	0.085	0.183
3. <i>baytopiorum</i>	50631	0.090	0.482	0.609
4. <i>bivonae</i>	54491	0.164	0.185	0.545
5. <i>bornmuelleri</i>	49687	0.073	0.250	0.231
6. <i>cilicicum</i>	33921	0.090	0.296	0.193
7. <i>kotschy</i>	45322	0.145	0.400	0.406
8. <i>macrophyllum</i>	48872	0.039	0.161	0.181
9. <i>speciosum</i>	43572	0.064	0.246	0.192
10. <i>umbrosum</i>	49688	0.300	0.104	0.174

Among the Turkish *Colchicum* species examined, the ones that have seeds and corms big enough to be used practically, and bear the highest amount of alkaloids are listed in Table 2.

In regard with the total alkaloid content calculated in terms of colchicine; the corms of the *C.baytopiorum* C.D.Brickell, *bornmuelleri* Freyn, *cilicicum* (Boiss.) Dammer and *kotschy* Boiss. and the seeds of the *C.baytopiorum*, *bivonae* Guss., *bornmuelleri*, *kotschy* and *speciosum* have either equal or higher amount of alkaloids when compared with medicinal *C.autumnale*.

On the other hand, the findings of the researches on the alkaloid content of the *Colchicum* species grown in Turkey are summarized below:

Corms: *C.cilicicum* has the richest alkaloid content in terms of colchicine and colchicoside; *C.bornmuelleri* and *C.kotschy* are richest in demecolcine.

Seeds: *C.baytopiorum* and *C.kotschy* have the richest colchicoside and demecolcine content. *C.bivonae* is the richest in demecolcine and *C.umbrosum* Steven has the richest colchicine alkaloid content.

The alkaloid content given in the tables varies according to the used method, the collecting times of the seeds or the corms and the climate conditions for each alkaloid in the species. Thus, the values given in the table should be considered as averages.

## EXPERIMENTAL

All the voucher specimens of Turkish *Colchicum* species used in determinations are deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE).

### Spectrophotometric determinations (3,4):

1. Powdered seeds and corms were percolated with EtOH. The dried extracts were taken up in water and extracted with petroleum ether and then ether. pH of the aqueous solution was first adjusted to 3 with 5 % HCl and extracted with chloroform (Neutral alkaloids extract). Then the acidic aqueous part was made alkaline (pH 8) with 10 % ammonia and extracted again with chloroform (Basic alkaloids extract).

The determinations were performed separately in neutral and basic extracts. Separation of the alkaloids was accomplished by preparative thin-layer chromatography on silicagel (Camac silicagel for TLC without binder D-O). The solvent system for Colchicine: EtOAc/IsoPrOH/Ammonia (25%) (80:15:5), Carr-Price Reagent ( $\text{SbCl}_3$  25+ $\text{CHCl}_3$  75); the solvent system for Demecolcine:  $\text{CHCl}_3/\text{Me}_2\text{CO}/\text{Ammonia}$ (25%) (50:50:1), Dragendorff Reagent (Munier-Macheboeuf).

Colchicine and demecolcine were eluted by MeOH and detected by UV at 352 nm using a Beckman Model 26 Spectrophotometer.

2. Low alkaloid content of the species having no economic importance with small seeds and corms maintained according to the above method were eliminated and then the rest 10 *Colchicum* species were investigated in methanolic extract with respect to their total tropolonic alkaloid content by a direct spectrophotometric method (Table 2) using Shimadzu UV-150-02 spectrophotometer.

### HPLC Determinations (4):

Powdered plant material (seeds and corms) was extracted with MeOH in a Soxhlet apparatus. The methanolic extract of the seeds and corms were quantitatively analysed by reversed phase HPLC using a Waters Liquid Chromatograph. Details of the analyses are given below:

Column:  $\mu$ -Bondapak C<sub>18</sub>(30 cmx3.9 mm), Pump: Model 6000 A, Detector: UV Model 440 (340 nm), Injector: Model U6 K Universal injector, Printer: YEW (Yokogawa Electronic Works), Model 3021 Pen Recorder.

Flow rate: 2 ml/min, Run time: 1 cm/min, Solvent system: Colchicine: MeOH/H<sub>2</sub>O (50:50); Demecolcine:MeOH/H<sub>2</sub>O (90:10).

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