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Research article

COMPARISON OF COLCHICINE CONTENT BETWEEN HYSTERANTHOUS AND SYNANTHOUS *COLCHICUM* SPECIES IN DIFFERENT SEASONS

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ABSTRACT

In order to compare of different phonological stages and seasonal changes of colchicine content between hysteranthous and synanthous colchicum species, amount of colchicine was determined in *Colchicum speciosum* Steven, *C. kotschyi* Bioss and *C. robustum* Stefanov, in different seasons, 2009–2010. The observations under wild conditions showed, that the leaves of appeared with flowers in the same stage of life cycle (synanthous) in *C. robustum*, while in case of *C. kotschyi* and *C. speciosum* flowers occurred first and leaves later, in another developmental stage (hysteranthous). Seed's colchicine content in *C. robustum*, *C. kotschyi* and *C. speciosum* was obtained as 1.28, 0.46 and 0.92 mg g^{-1} dry weight, respectively. Corm's colchicine content was higher in *C. speciosum* than the other species in all seasons. The highest colchicine content of corm in *C. speciosum* was obtained in winter and autumn (2.17 and 2.13 mg g^{-1} dry weight, respectively), while in *C. robustum* and *C. kotschyi* it was found in autumn, 0.49 and 0.77 mg g^{-1} dry weight, respectively. The lowest colchicine content of corms was obtained in summer, when the corms were dormant before flowering stage, in *C. speciosum* and *C. kotschyi*, 0.131 and 0.0058 mg g^{-1} dry weight, respectively, whilst in *C. robustum* obtained in winter, 0.08 mg g^{-1} dry weight, synchronous to flowering and vegetative growth.

KEYWORDS: Colchicine content, *Colchicum kotschyi*, *C. speciosum*, *C. robustum*, Flowering stage, Hysteranthous, Root activity, Synanthous, Seasonal changes.

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INTRODUCTION

The genus *Colchicum* belongs to the family Colchicaceae, which comprises of 19 genera, and 225 species (Nordenstam, 1998). Plants of the genus *Colchicum* have been known for more than 2000 years for their marked beneficial and poisonous effects (Brickell, 1984). The modern medicine uses *Colchicum* as a source of therapeutically active alkaloids called colchicinoids. One of the most abundant alkaloid - colchicine, is known to have cancerostatic, antirheumatic, antimitotic, antiinflammatory, cathartic and emetic effects. It is also applied in plant breeding to induce polyploids (Komjatayova *et al.*, 2000; Frankova *et al.*, 2005). In addition to the genus *Colchicum*, colchicine was reported from species belongs to *Merendera* and *Gloriosa* genera, which belonging to the Colchicaceae family (Nordenstam, 1998).

Very little is known about the factors interfering with the biosynthesis of colchicine-like alkaloids. Results obtained by Sütlüpinar *et al.* (1988), indicated that the composition of tropolone alkaloids differs in different parts of the plants and varies during the different growth stages (Sütlüpinar *et al.*, 1988). Presence and concentration of colchicine is determined by a variety of environmental factors including season (Vicar *et al.*, 1993; Poutaraud and Girardin 2002; Alali *et al.*, 2006) and resource availability (Hayashi *et al.*, 1988; Pouraraud and & Girardin, 2005; Mróz, 2008) as well as genetic variations between populations and individuals (Poutaraud and Champay, 1995). Also colchicine content varies among different organs of the plant body (Sütlüpinar *et al.*, 1988; Alali *et al.*, 2004; Alali *et al.*, 2006).

Among all species of *Colchicum*, *C. autumnale* is the best source for colchicine. The richest plant parts in colchicine are the corms and seeds. *C. autumnale* seeds contain 0.6-1.2%, while corms contain up to about 0.6%. Seeds are mainly used by the pharmaceutical industry for the extraction of colchicinoids (Trease & Evans, 1983). The content of colchicine alkaloid in corms, stems, leaves, and flowers of *C. cilicicum* were 0.05%, 0.01%, 0.01% and 0.20% (g% dry weight), respectively (Sütlüpinar *et al.*, 1988). In another study by Alali *et al.* (2004), *C. stevenii* corms, flowers and leaves were reported to contain 0.17, 0.12 and 0.20 (wt/wt) g%, respectively, while *C. hierosolymitanum* corms and flowers were found to contain 0.13 and 0.09 (wt/wt) g%, respectively. Ondra *et al.* (1995), assayed corms of seven Turkish *Colchicum* species; namely: *C. macrophyllum*, *C. turcicum*, *C. cilicicum*, *C. kotschyi*, *C. bornmuelleri*, *C. speciosum* and *C. triphyllum* for their colchicinoid alkaloids. Colchicine content was found to be 222.3, 323, 300, 1058, 3063, 4245 and 958 μg g^{-1} dried drug, respectively.

Colchicine variation in different organs of plant and during different growth stages has been studied by researchers. Colchicine and demecolcine were determined in raw and dried leaves, stems, mother and daughter corms of *C. autumnale* in four stages of its ontogenesis by Vicar *et al.* (1993). They found that colchicine content in raw material varies during plant growth. Colchicine content in *C. brachyphyllum* and *C. tunicatum*, was determined during different growth stages by Alali *et al.* (2006). Underground parts in both species and during different growth stages, always showed higher colchicine content than the above ground parts. In *C. brachyphyllum*, total colchicine content of underground parts during flowering stage was found to be about 0.15% (wt/wt), while that of aerial parts was only about 0.04% (wt/wt). In *C. tunicatum*, total colchicine content of underground parts was found to be 0.12% (wt/wt) and 0.13% (wt/wt) during flowering and vegetating stages, respectively, while that of aerial parts was only about 0.04% (wt/wt) and 0.02% (wt/wt), respectively (Alali *et al.*, 2006).

Generally, geophytes are plants that survive by subterranean storage organ with renewal buds (Raunkiaer, 1934). They divide into two groups – synanthous and hysteranthous one. The leaves of synanthous geophytes coexist with flowers in the same stage of life cycle. In case of hysteranthous plants flowers occur in the first and leaves later, in another developmental stage (Dafni *et al.*, 1981). A special case is the

hysteranthous plant *Colchicum tunicatum* which perceive the photoperiodic signal when the dry bulb lies well below the soil surface (Halevy, 1990). *C. speciosum*, *C. kotschyi* Boiss, and *C. robustum* stefanov, are three wild growing Iranian *Colchicum* species (Presson, 1992). *C. speciosum* Steven and *C. kotschyi* Bioss are hysteranthous but *C. robustum* is a synanthous species (Presson, 1992).

So far no study has been performed on colchicine content variation between synanthous and hysteranthous *Colchicum* species in different seasons, thus the aim of this study was to evaluate phenological changes and their relationship with corm and seed colchicine content variation among three Iranian native *Colchicum* species, under their habitat conditions.

MATERIAL AND METHODS

Plant Material

The corms of three wild *Colchicum* species were collected in different seasons (spring, summer, autumn and winter during 2009–2010, and seeds were collected in spring 2010. Corms and seeds of *C. speciosum*, *C. robustum* and *C. kotschyi* were collected from Khalkhal-Asalem road, Ardabil province, at an altitude of 940m in Iran, Babaaman Mountain, North Khorassan Province, at an altitude of 1091m in Iran and Noghondar valley near Mashhad, Razavi Khorasan province, at an altitude of 1400m in Iran, respectively. The collected materials of three species were identified by Mohammad Reza Joharchi, Ferdowsi University of Mashhad Herbarium (FUMH). Voucher specimens of *C. kotschyi* (Herbarium Number: 39516), *C. robustum* (Herbarium Number: 39519) and *C. Speciosum* (Herbarium Number: 39531) were registered. These are kept in the herbarium of FUMH.

Recording developmental stages

To study the plant phenology in wild conditions observations were carried out for three species from three different locations during 2009–2010. Observations were including of developmental stages such as beginning of flowering, peak flowering time, root formation time, beginning of vegetative growth, fruiting and capsule formation and daughter corm formation in wild conditions.

Extraction and Isolation

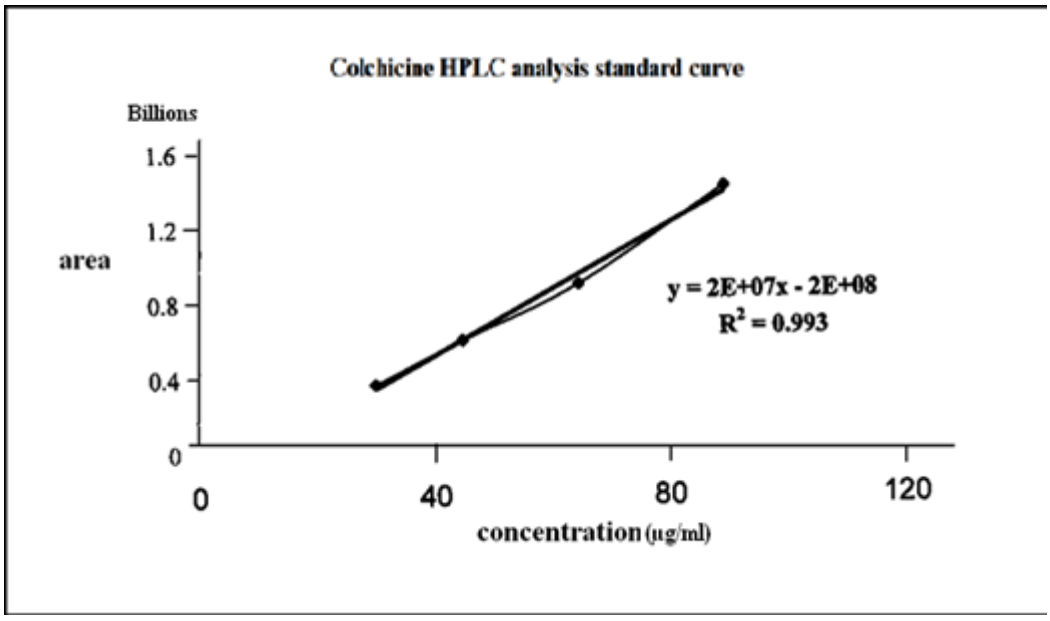
The methods described by Rosso and Zuccaro (1998) and Alali *et al.* (2006), were adopted with some modifications. Acetonitrile, methanol and other reagents were of chromatographic grade and prepared from Panreac (Spain). Reference standard of colchicine was prepared from USP.

The corms were sliced into small pieces and air-dried at room temperature together with the seeds. After drying, exact weight of 2 g of corms (collected in different seasons) and 2 g of seeds of three *Colchicum* species were grounded to powder in a laboratory mill and then used for extraction. Powdered material placed into 250 mL Erlenmeyer flasks and extracted with 100 mL of methanol in 35^{o}C for 1h with ultrasonic apparatus. Afterwards, plant residues were filtered through Wattman filter paper and the filtrates were saved. Then plant residue was transferred into Erlenmeyer flasks again and extracted with 50mL of methanol in 35^{o}C for 30min with ultrasonic apparatus and then filtered. Plant residues were washed with 10 mL of methanol and then filtered. The collected filtrates and washes were combined and transferred into a 250 mL separatory funnel and extracted with petroleum ether (30 mL × 3) with frequent shaking for 30 min in order to remove non-alkaloid substances. 10 mL of distillate water was added each time for better separation and creation of two separate phases. The resulting methanolic phase was transferred to an empty separatory funnel and extracted with chloroform (30 mL × 3) for 10 min. The chloroform phases obtained from three stages were collected and Sodium sulphate Anhydrous was added to the chloroformic solution for dewatering of it and then filtered through filter paper. The chloroformic extract was dried in vacuum and then dissolved in 5 ml of HPLC

grade methanol and injected to the HPLC instrument. Injection volume at 50 µl, room temperature, detection at 243 nm. All analyses were done in duplicate.

HPLC instrument was Knauer ® (Germany) equipped with auto sampler and column was Bondapak C18 (Technochrom) micrometer particles and 4.9 mm id and 250 mm in length. An UV detector K-2501 and a dynamic mixing chamber was employed. Mobile phase system consisted from phosphate buffer pH=6 and acetonitrile (77: 23). For preparation of phosphate buffer 800 mg of NaH_2Po_4 and 200mg of Na_2HPo_4 were dissolved in 1000mL of HPLC grade water and the pH was adjusted on 6. The flow rate was adjusted to 2mL/min and detection was performed at a wavelength of 243nm. The stock solution of colchicine standard was prepared by accurately weighing of colchicine reference standard and then diluted using HPLC grade methanol to construct calibration curve of six –points (30, 50, 75, 90, 100 and 120 ppm). Figure 1 shows colchicine HPLC analysis standard curve.

Figure. 1. Colchicine HPLC analysis standard curve



RESULTS AND DISCUSSION

Developmental stages

Table 1 shows, beginning time of developmental stages in three *colchicum* species under their habitat conditions. The results showed that flowering started sooner in *C. Speciosum* (end of August) and *C. kotschyi* (middle of September) than to *C. robustum* (end of January). Fruiting and capsule formation started later in *C. robustum* (middle of April) than to *C. speciosum* (beginning of April) and *C. kotschyi* (end of March). In all species root activity got initiated in middle of autumn (Table 1).

Observations showed that *C. kotschyi* and *C. speciosum* were hysteranthous geophyte (flowers develop first and leaves later) and autumn-flowering species but *C. robustum* was a synanthous geophyte (leaves coexist with flowers in the same stage of the life cycle) and winter-flowering species. This type of obviously hereditary phenological behaviour is rather the rule in the genus *Colchicum*, in contrast to the onset of leaf growth which seems to be largely environmentally triggered (Burtt, 1970; Gutterman and Boeken, 1988; Persson, 1999).

Table 1. Beginning of developmental stages of three *colchicum* species under natural conditions

Different Species	Beginning of flowering	Pick time of flowering	Root formation	Vegetative growth	Fruiting and capsule formation	Daughter corm formation
C. speciosum	E[a]-Aug	E-Sep	E-Oct	E-Mar	B-Apr	E-May
C. kotschyi	M-Sep	B-Oct	B-Nov	B-Feb	E-Mar	M-May
C. robustum	E-Jan	M-Feb	B-Dec	B-Feb	M-Apr	B-May

Notes: [a] B, M and E indicate the beginning, middle and end of each month, respectively

Colchicine content

The results of HPLC analysis of plant extracts are summarized in table 2. The level of colchicine varies in different seasons as well as species and plant parts. Seed's colchicine content in *C. robustum* was higher than the other species. Seed's colchicine content in *C. speciosum*, *C. kotschyi* and *C. robustum* was 0.92, 0.46 and 1.28 mg g^{-1} dry weight (DW), respectively (table 2). The amounts of corm colchicine in *C. speciosum* were higher than the other species in all seasons. Among different seasons the highest colchicine content of corm in *C. speciosum* was obtained in winter (2.17 mg g^{-1} DW), while in *C. robustum* and *C. kotschyi* it was found in autumn, 0.49 and 0.77 mg g^{-1} DW, respectively. The lowest colchicine content of corm was obtained in summer in *C. speciosum* and *C. kotschyi* was found to be about 1.31 and 0.058, respectively, while *in C. robustum* it was in winter, 0.08 mg g^{-1} DW.

Corm's colchicine content in *C. speciosum* and *C. kotschyi* (as hysteranthous species) in autumn and winter were higher than to spring and summer, while in *C. robustum* (as a synanthous species) the highest corm colchicine content was obtained in autumn. The lowest colchicine content in *C. kotschyi* and *C. speciosum* was obtained in summer, whilst in *C. robustum*, it was observed in winter.

Colchicine content in different species varies considerably during different seasons. Matching of the table related to developmental stages with seasonal variation of colchicine content indicates that corm colchicine content in the three *colchicum* species studied was high in autumn (at the time of root activity). The lowest colchicine content of corm in *C. speciosum* and *C. kotschyi* (as hysteranthous species) was observed when the corms were dormant, while in *C. robustum* (as a synanthous species) it was obtained during flowering and vegetative stages. During flowering stage and in the absence of leaves, the only source of colchicine in flowers could be due to the translocation of colchicine from corms and this may explain the slightly low corm colchicine content at flowering stage (Al-Fayyad *et al.*, 2002).

Seed colchicine content in *C. robustum* was higher than those of the other species. Previously reported that the amount of seed alkaloid and colchicine content is more in unripe seed and declines as the seeds mature (Poutaraud and Girardin, 2003; Alali *et al.*, 2006). Since In the present study, the capsules and seeds of *C. speciosum* and *C. kotschyi* were formed sooner than those of *C. robustum* so it seems that, less colchicine content in *C. speciosum* and *C. kotschyi* seeds had been due to more mature their seeds than those of *C. robustum*.

Table 2. Mean Colchicine content of different organs of the three *Colchicum* species (mg g^{-1} dry weight) during different seasons.

Species				
Season	organ	*C. speciosum*	*C. robustum*	*C. kotschyi*
Winter	corm	2.17 ± 0.04[a]	0.08 ± 0.002	0.60 ± 0.03
Spring	corm	1.47 ± 0.03	0.21 ± 0.003	0.50 ± 0.03
	seeds	0.92 ± 0.02	1.28 ± 0.02	0.46 ± 0.04
Summer	corm	1.31 ± 0.02	0.13 ± 0.002	0.06 ± 0.003
autumn	corm	2.14 ± 0.04	0.49 ± 0.006	0.77 ± 0.03

Notes: [a] Colchicine content is expressed as mass of colchicine in 1 gram dry weight ± standard deviation, derived from the average of two extraction replicates, each run in duplicate

Alkaloids are responsible for the plant adaptation to its environment. It is known that alkaloids are efficiently used as defensive agents and they may be moved around the plant to those parts needing greater protection during growth and development (Harborne, 1997). As part of their defences against herbivores, many geophytes are toxic and unpalatable, or have developed different physical defences against herbivores (Lovegrove & Jarvis, 1986; Go´mez-Garcı´a *et al.*, 2004). This is the case of different plant species of *colchicum*, which contain colchicine.

CONCLUSION

In conclusion, what the results suggest is that the highest corm colchicine content in the three species was found in autumn (the period of root activity). Thus the corms of these three species are better to be collected in autumn from their local habitats, to ensure that maximum of colchicine is achieved. The lowest corm colchicine content in *C. robustum* (as a synanthous species) was observed in winter (at flowering stage) whereas in *C. speciosum* and *C. kotschyi* (as hysteranthous species) in summer, when the corms are dormant. However, more species of *colchicum* need to be examined to determine a stronger relationship between developmental habit (specially flowering habit) and colchicine content.

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