

# Anatomical and phytochemical studies and ethnomedicinal uses of *Colchicum autumnale* L.

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### Correspondence

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**Ethnobotany Research and Applications 25:6 (2023)** - http://dx.doi.org/10.32859/era.25.6.1-9 Manuscript received: 31/12/2022 – Revised manuscript received: 15/01/2023 - Published: 15/01/2023

# **Notes on Ethnobotany**

# Abstract

*Background*. In the world, great importance is attached to the intensification of plants. To date, most medicinal plants are only partially used. In case of *Colchicum autumnale* L. currently mainly the corms are used for the extraction of alkaloids. The purpose of this study was to investigate the intensification of the use of this perspective plant reported from the study area. This article examines the anatomy and phytochemistry of leaf and corm of *C. autumnale*, which is grown as a medicinal crop in Uzbekistan.

*Methods*: The leaves were fixed in 70% ethanol according to the generally accepted method; transverse and paradermal sections were examined on the fixed material. When studying the anatomical features of the leaf, preparations was formed by manually cutting a transverse section of the leaf were observed by a MOTIC V1 microscope. The slides were stained with glycerin jelly having safranin. Cells and tissues were measured using a micrometer MOB-15. To determine the amount of alkaloids contained in the leaves and in corms, a chemical analysis was carried out in the laboratory of alkaloids of the Institute of Chemistry of Plant Substances.

*Results*: On the transverse section of the stomata of the leaves of *Colchicum autumnale* anomocytic type, 2-3 rows of parenchyma and palisade cells, the characteristic size of the stomata. The root structure of *C. autumnale* consists of 4-5 rows of corolla, 4 protoxylems and 1 metaxylem, and the presence of rhomboid calcium oxalate crystals in its parenchyma is a characteristic feature of the species. As a result of studying the chemical composition of the plant in the conditions of the Tashkent region, it was observed that the amount of alkaloids in the aerial part of the colchicum, in the leaves was 0.4% and in the corm 0.14%.

*Conclusion*. The current work sought to resolve taxonomic issues involving species that share the majority of their physical characteristics and to clarify relationships between important taxa by utilizing leaf epidermal characteristics. As a result of chemical analysis, it was revealed that the amount of alkaloids in the leaves of *Colchicum autumnale*, introduced in the conditions of the Tashkent region (Uzbekistan), is higher than in natural regions. This proves that in the conditions of Uzbekistan it is possible to grow *Colchicum* and obtain high-quality raw materials.

Key words: autumn crocus, meadow saffron, anatomy, phytochemistry, ethnomedicine.

# Background

In the world, great importance is attached to the continuously intensifying use of plants. Most medicinal plants are however only partially used. In case of *Colchicum autumnale* L. currently mainly the corms are used for the extraction of alkaloids. The purpose of this study was to investigate the intensification of the use of this perspective plant reported from the study area. This article examines the anatomy and phytochemistry of leaf and corm of *C. autumnale*, which is grown as a medicinal crop in Uzbekistan.

The name *Colchicum* L. is associated with the name of the Colchis valley on the Black Sea coast (Rodnan, Benedek 1970). The genus *Colchicum* was first described by C. Linnaeus, who combined 45 species into this genus (Takhtadzhyan *et al.* 1982). Later, Stefanov devoted to this genus, describes 64 species in his monograph (Stefanov 1926). In another monograph, Persson increased the number of species to 99, describing a number of new species (Persson 2007).

Autumn-flowering, hysterantous, species of the genus *Colchicum* are mainly distributed along the Mediterranean Sea, 39 species of the genus exists in the European flora (Brickell 1980), 50 species in the Turkish flora and 31 species in Greece, 11 species grow on the territory of the CIS countries and two species of the genus grow in Central Asia (Buyankin, Nikolskaya 2019; Sodikov, Yusupov 1959; Karlik *et al.* 2020; Milovic 2017). Only the species *C. autumnale* is widely distributed in Asia and Africa, reaching the south of Scandinavia, the coast of the Baltic Sea, and the south of the British Isles. In the Ukrainian Carpathians, this plant is common in different ecological latitudes, from lowland to highland belts. While analyzing the soil during its growth stage it was recorded that the acidity level is high, that is, pH 3.7-5.0. According to the mechanical composition, the soils are mostly sandy (Oganezova 2019). Also it grows in the west and south of Central Europe - France, Belgium, south of Germany, Denmark, south of England, Italy, Bulgaria, Yugoslavia, in the east - Poland, Romania, Czechoslovakia, Hungary. It is listed in the Red Book as an endangered species in Ukraine, Ireland, Albania, Latvia, Luxembourg, Belarus, Estonia, Great Britain, the Netherlands, and Germany (Jung *et al.* 2011).

In recent years, the number of ethnobotanical studies in Uzbekistan is increasing (Khojimatov *et al.* 2020; Tayjanov *et al.* 2021). This scientific research work can serve as a basis for effective use of medicinal plants.

# **Materials and Methods**

#### **Object study**

In Uzbekistan, *C. autumnale* L. is an introduced species imported from Europe for cultivation, initially by Yu. Murdukhaev, and later in 2017 by Makhkamov from Poland. At present, *C. autumnale* is in the collection of the Laboratory for the Introduction of Medicinal Plants of the Tashkent Botanical Garden, in the Botanical Garden of the National University of Uzbekistan and in the experimental site of the SUE Extension Center of the Tashkent State Agrarian University.

The object of study , *C. autumnale* L., is a perennial hysterantous geophyte of the Colchicaceae family (Fig. 1). The stem is short; bulb is elongated, up to 7 cm in height and up to 3 cm in diameter. The leaves are erect, glabrous, fleshy, shiny, and oblong-lanceolate. The flowers are large, pink or pale lilac, the peduncle is long, the lower part of which is located on the ground. The fruit is a three-celled, oblong-ovoid capsule; the seeds are almost spherical in shape and reach 2-2.5 cm in diameter. Flowering period of the species is from August-September. During the flowering stage the plant does not have leaves. Pollinated seed pods overwinter underground (Akopov 1986).



Figure 1. C. autumnale in the vegetative and generative phases.

#### Leaf and root anatomy

For preparation of samples to leaf and root anatomical study the samples of leaves and roots were fixed in 70% ethanol according to the standard procedure, and cross sections were studied on the fixed material (Trankovsky 1979).

The leaf was fixed in 70% ethanol according to the generally accepted method; transverse and paradermal sections were examined on the fixed material (Trankovsky 1979). When studying the anatomical features of the leaf, preparations was formed by manually cutting a transverse section of the leaf were observed by a MOTIC V1 microscope. The slides were stained with glycerin jelly having safranin. Cells and tissues were measured using a micrometer MOB-15. Quantitative measurements of a number of leaf characteristics: the height of epidermal cells, the thickness of the epidermal wall, the length and width of stomata, the thickness of the mesophyll, the height and width of palisade cells were investigated using the generally accepted method (Dospehov 1985). Statistical data processing was examined on a personal computer (MS Excel) according to standard methods. Micrographs were taken with a digital camera and mathematical analysis was performed using a microscope MOTIC.

# Phytochemistry of leaves and corms

To determine the amount of alkaloids contained in the leaves and in corms, a chemical analysis was carried out in the laboratory of alkaloids of the Institute of Chemistry of Plant Substances.

Extraction of the leaves of *Colchicum autumnale*. 100 g of dry crushed raw material was extracted with 80% ethanol. The solution was allowed to cool for a day and poured into a separate container. The draining process was repeated eight times continuously. Alcohol was distilled off from the obtained water-alcohol extract. The remaining aqueous solution was partitioned between chloroform and 5% sulfuric acid. Isolation of the alkaloid with acid was continued until a negative reaction to the alkaloid appeared. The acidic solutions were combined and made basic with ammonia. Alkaloids were extracted with chloroform for a while. The process continued until the solution became thick.

#### **Data Collection**

We obtained ethnomedicinal data from Avicenna and popular journal papers, monographs, books written in Uzbek and Russian, English and also ours own research focused on the traditional use of medicinal plants.

## **Results and Discussion**

#### Anatomical structure of the leaf and root

The anatomical structure of the leaves and roots of the plant was analyzed using microscopic techniques. On a transverse section of *Colchicum autumnale* L. leaf, it is covered on both sides with a single layer of isodiametric rectangular epidermis (Fig. 2).

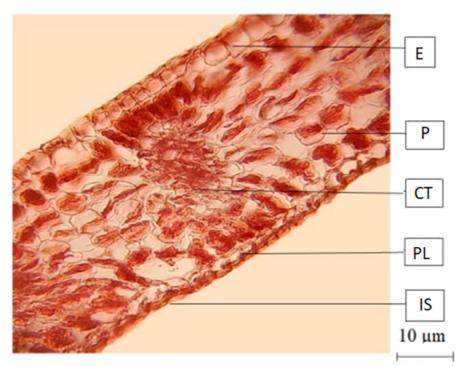


Figure 2. Transverse section of leaf of *C. autumnale* (E) Epidermis, (P) Parenchyma, (CT) Conductive tubules, (PL) Palisade, (IS) Intercellular spaces.

The outer epidermis is covered with a thin layer of cuticle. The epidermis has anomalies of the anomocytic type, 25.5  $\mu$ m in size (Fig. 2, Table 1). The mesophyll is located between the upper and lower epidermis and consists mainly of parenchyma and palisade cells. The parenchyma and palisade cells are thin-walled, rounded, angular, elongated, filled with numerous chloroplasts, crystals are also present. Between the cells of the parenchyma and the palisade there are large intercellular spaces that occupy a larger volume than the cells.

Table 1. Anatomical indicators of the structure of leaves of *C. autumnale* 

cators of the structure of leaves of <i>C. autumnale</i>		
Indicator	μm	
Height of the upper epidermis	14,2±0,4	
Height of the lower epidermis	10,1±0,3	
Length of stomata	25,5±0,7	
Width of stomata	22,5±0,7	
Thickness of external wall	3,8±0,1	
Thickness of mesophyll	220,7±2,3	
Height of parenchymal cell	28,5±0,7	
width of parenchymal cell	7,8±0,3	

The delimitation of the species under study here was greatly helped by the micromorphological analyses of foliar anatomy. For instance, the epidermal leaf anatomy of the chosen species revealed both qualitative (e.g., epidermal cell shape, lobes per cell, wall pattern, type of stomata, shape of stomata, shape of stomatal pores, shape of guard cells, inner margin of outer stomatal ledge, ornamentation of outer stomatal ledge) and quantitative (e.g., number of epidermal cells, epidermal cell size).

On the transverse section of the stomata of the leaves of *C. autumnale* anomocytic type, 2-3 rows of parenchyma and palisade cells, the characteristic size of the stomata (Fig. 3, 4).

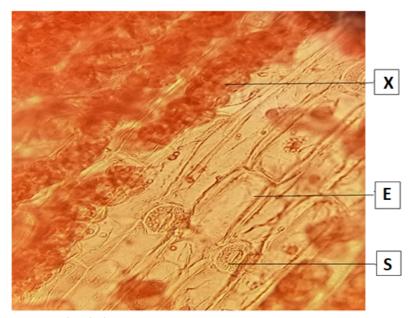


Figure 3. Paradermal section of leaf of *C. autumnale* (X) Chloroplasts, (E) Epidermis, (S) Stomata

A single row of epidermis is visible on a transverse section of the root of *C. autumnale* (Fig. 5, Table 2). The epidermis is covered with a thin cuticle. At the base of the epidermis is the exoderm, consisting of 1-2 rows. After the exoderm, there is a 4-5-row cortex, consisting of large parenchyma cells. At the base of the nucleus there is one row of rectangular endoderm. One row of pericycle is visible under the endoderm.

The Xylem consists of 4 protoxylems and 1 metaxylem. The type of conductive tissue is the collateral. The parenchyma contains diamond-shaped calcium oxalate crystals (Kahraman, Celep 2010; Akan, Satil 2005) (Fig. 6). On epidermis, exodermis, and endodermis, a striking variation in epidermal cell size and density was seen.

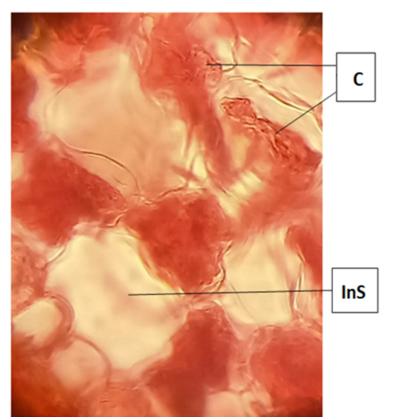


Figure 4. Cross section of leaf of *C. autumnale* (K) Crystal, (InS) Intercellular spaces

Table 2. Anatomical indicators of the structure of the root of <i>C. autumnale</i>		
	Indicator	μm
	Height of epidermis	11,3±0,3
	Height of exodermis	9,8±0,3
	Height of endodermis	3,8±0,1
	Width of bark	222,7±2,3
	Height of parenchymal cell	26,5±0,7
	Width of parenchymal cell	7,8±0,3

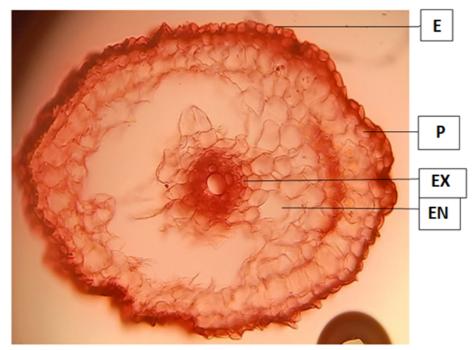


Figure 5. Transverse section of the root of *C. autumnale* (E) Epidermis, (P) Parenchyma, (EX) Exoderm, (EN) Endodermis

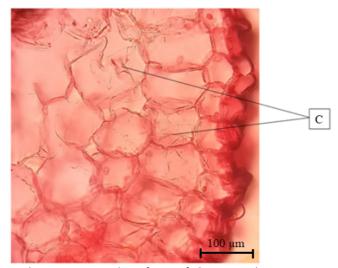


Figure 6. Appearance of crystals on a cross section of root of *C. autumnale* 

The root structure of *C. autumnale* consists of 4-5 rows of corolla, 4 protoxylems and 1 metaxylem, and the presence of rhomboid calcium oxalate crystals in its parenchyma is a characteristic feature of the species.

#### Phytochemistry of leaves and corms

As a result of studying the chemical composition of the plant in the conditions of the Tashkent region, it was observed that the amount of alkaloids in the aerial part of the *Colchicum autumnale* L. in the leaves was 0.4% and

in the corm 0.14%. Kefi (2018) studied the alkaloids of *C. autumnale*, compared with intergrowths grown in vitro. According to him, the amount of alkaloids: in leaves 0.15-0.4%, in corms 0.1-0.6%, in seeds 0.5-1.2%, in fresh flowers 1.2-2.0%.

The result of study showed the presence of a large amount of alkaloids in the raw material of a plant grown in natural conditions. Kabaloeva (2013) determined the content of colchicine and colchamine in homeopathic matrix tinctures of *Colchicum*. According to them, the amount of alkaloids in the corm was 0.086%. Gribok *et al.* (2007) studied the alkaloid colchicine in leaves, stems, buds, flowers, and bulbs of *C. autumnale* in different periods of vegetation. According to their data **at the beginning of the growing season** the alkaloid content was: in corms - 0.031%, in leaves 0.612%; in the phase of corm formation: in the corm 0.118%, in the leaves 0.157%, in the stems 0.293%; **at the end of the growing season**: in corms 0.09%, in leaves 0%; **in the budding phase**: in corms 0.066%, in buds 0.802%; **in the flowering phase**: in corms 0.046%, in flowers - 0.511%.

If we consider the large mass of the above-ground part of the plant and the high content of alkaloids, then this indicates the possibility of obtaining leaf raw materials from the first years of growing this plant under introduction conditions. This makes it possible to intensify the use of the introduced plant.

#### **Ethnomedicinal uses**

*Colchicum autumnale* L. carefully used for medicinal purposes due its poisonous alkaloid. In ethnomedicine, this plant has long used to treat various diseases. For example Abu Ali ibn Sina used *C. autumnale* as an ingredient in the preparation to treat gout, diseases of the large intestine (kulinja) and various other diseases also as a sedative (Khodjimatov 1989) of various ointments, solutions also porridges (Ibn Sino 1980a, b).

According to Ibn Sina *C. autumnale* is a drug of choice for arthralgia in the form of Zimad (Baitar 1999; Anonymous 2012). *C. autumnale* was useful joint pain, gout and ischemia (Ibn Sino 1980a, b).

*C. autumnale* has been used as an ingredient of Medicinal porridge, known under the name "amiri". It is used for uremia, back pain and weakness of the kidneys, kidney-stones (Ibn Sino 1980b). *C. autumnale* has also been used in the treatment of "frog" disease - a kind of hard bump under the tongue (Ibn Sino 1980a), and to treat pain in the knees and back, with paralysis and paralysis of the facial nerve (Ibn Sino 1980b).

*C. autumnale* is one of the known drugs of Unani medicine. Its adverse effects on the stomach and liver has prompted Unani physicians to use it cautiously (Baitar 1999).. It is also called hermodactyle in Greek, which means fingers of Hermes, due to its flowers looking like "fingers of Hermes" (Afsahul *et al.* 2020).

In Unani Tibb it used for removing joint pains, backache and gout, etc. It is accepted largely by Unani and Ayurvedic physicians as a traditional healer of internal injuries (Akram *et al.* 2012).

The flowers and corms are used medicinally for the treatment of arthralgia, gout, sciatica etc. (Khan, Muhiti 2014). These effects occur due to its analgesic, resolvent and de-obstruent properties and hot and dry temperament which may be attributed due to the chemical constituents mainly colchicine and demo colchicine present in the corms (Afsahul *et al.* 2020).

Herbal raw materials and tinctures are described in the homeopathic pharmacopoeias of Germany, France, Great Britain, the USA and India (Kabaloeva 2013). A tincture prepared from the corms is used in homeopathy for the following diseases: gout, rheumatism of the joints, cystitis, urolithiasis, neuralgia, circulatory disorders, respiratory diseases, as a remedy for inflammation of the digestive and respiratory organs (Kabaloeva 2013).

*C. autumnale* has been used as an ingredient of Gouticin. Gouticin tablets were found effective for treatment of gouty arthritis (Akram, Mohiuddin 2010).

*C. autumnale* has a slight toxic effect. As a pain killer *C. autumnale* gives relief in all types of muscular, joint and gastric pains. *C. autumnale* relieves burning muscular tissues, periosteum and synovial membranes of joints. *C. autumnale* is a beneficial treatment for the foot palm burning. *C. autumnale* has positive effects on the efficiency of the sex organs. *C. autumnale* has also been used for enlarging of penis. *C. autumnale* is used to treat several kinds of cancer tumor and unnecessary access in growth of cells (Anonymous 2012).

The alkaloid (Colchamine) is used in the treatment of skin cancer and chronic leukemia, and colchicine is used in the treatment of patients suffering from backache, gout, and sometimes neuralgia (Kholmatov, Ahmedov 1996). Colchicine is employed in animal studies to explore wound healing and embryonic growth (Akram *et al.* 2012).

Other Colchicum species are also important in ethnomedicine. Colchicum aitchinsonii and Colchicum luteum seeds are similarly used to relieve pain and inflammation and in plant breeding work to induce doubling of chromosomes. The corms are dried and grinded to powder form then it is mixed with Ghee and used externally for lessening inflammation and pain. The local people mix the corm of Colchicum luteum with egg and fry it with cow's ghee. It is given to aged men and women in the evening before going to bed and is considered as remedy for joints pain. The plant is used for bronchial diseases, and the cure of rheumatism. The plant is used as blood purifier, laxative, aphrodisiac and for joints pain. Fresh ground corms are taken orally by nursing mother to improve lactation. Dried ground seeds are taken orally to improve digestion and to treat anorexia. Dried ground corms are mixed in food and taken orally with water to treat knee pain. Very small amount of powder is given by Hakims (specialist people) in local oils as aphrodisiac and in joint pains, spleen & liver diseases. The powdered bulb used as antidiabetic, blood purifier, purgative, for flatulence, and liver disorder. The dried and powdered corm is used for gout. It is also used as carminative in low doses. The powdered herb is used orally for joint pains and arthritis. The plant extract is useful in digestive and gastric problems and also efficacious in liver and spleen disorders. Used to treat anorexia, improve digestion, as galactagogue, for keen pain, abdominal pain and as body tonic. For cough, dandruff, fever, gout, liver problems, rheumatism, gynecological problems, skin diseases, spleen problems, constipation, stomach (Sher *et al.* 2021).

# Conclusion

The current work sought to resolve taxonomic issues involving species that share the majority of their physical characteristics and to clarify relationships between important taxa by utilizing leaf epidermal characteristics. As a result of chemical analysis, it was revealed that the amount of alkaloids in the leaves of *Colchicum autumnale*, introduced in the conditions of the Tashkent region (Uzbekistan), is higher than in natural regions. This proves that in the conditions of Uzbekistan it is possible to grow Colchicum *autumnale* and obtain high-quality raw materials.

# Declarations

List of abbreviations: Not applicable.

Ethics approval and consent to participate: Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and materials:** All the data are presented in tables and figures in the manuscript and are available with the corresponding authors.

Competing interests: The authors declare that they have no competing interests.

**Funding:** Ministry of Innovative Development of the Republic of Uzbekistan supported this research project (no. AL 2021090820).

**Authors' contributions:** SB, TM, MZ collected, analyzed the data and drafted the manuscript. RB, SB, TM, MZ and AY advised, reviewed, and approved the final manuscript.

## Acknowledgements

TM would like to thank Ministry of Innovative Development of the Republic of Uzbekistan. We are also indebted to thank the anonymous reviewer for reading the manuscript and providing important comments.

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