Acta Poloniae Pharmaceutica – Drug Research, Vol. 76 No. 6 pp. 919–938, 2019
DOI: 10.32383/appdr/111762
ISSN 0001-6837
Polish Pharmaceutical Society

REVIEW

A REVIEW ON *CYCLAMEN* SPECIES: TRANSCRIPTION FACTORS VS. PHARMACOLOGICAL EFFECTS

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Abstract: The mechanism of floral mutation including double flowers in plant species can be explained by the ABCDE model which represents the relationship between MADS-box transcription factor genes and floral morphogenesis. The ornamental importance of *Cyclamen* led to the creation of double-flowered cultivars caused by the repression of AG-like gene expression in whorl 3. Modifications in these genes also influence the accumulation of different bioactive compounds depending on species and/or variety. In antiquity, cyclamen plants were recognized for their therapeutic qualities and later gained importance due to their content in saponins, which have been reported to exhibit anti-cancer, anti-inflammatory and antibacterial effects, and also to alleviate symptoms in acute rhinosinusitis patients. Furthermore, the extracts and isolated compounds are used as treatments in a wide range of diseases. In this review, we describe the transcription factors and their role in the development and ABCDE model formation of organs which led to the development of double-petal and fragrant varieties. Additionally, we describe the potential mechanisms of action underlying the therapeutic effects of saponin extracts against cancers and inflammatory disorders and their potential as a pharmacological agent in clinical studies.

Keywords*:* ABCDE model, cyclamin, MADS-box genes, medicinal properties, saponins, volatile compounds

The genus *Cyclamen* takes its name from the spherical tuber or the spiraled peduncle, shape that in Greek is called *kyklos*. Romans called it *cyclaminis,* and later, around the year 1700, the French botanist Tournefort named it *Cyclamen* (1). The 24 species (2) comprised in the genus *Cyclamen* are part of order *Ericales*, family *Primulaceae*, subfamily *Myrsinoideae* and are distributed in and around the Mediterranean region. Some of the species are frost resilient, some of which can bloom from late summer until late spring (Fig. 1).

In the genus *Cyclamen*, only *Cyclamen persicum* Mill. has been significantly used commercially. Even though wild *C. persicum* Mill. plants usually bloom from winter to spring, the production period of commercially-grown plants depends on climate conditions and demands in each country (3). Genus *Cyclamen* has been bred for more than 150 years. Initially, the focus was on plants with large flowers and shifted towards the selection of wide ranges of colors, based on the white and pink seen in wild *C. persicum* Mill.

At the same time, different petal shapes appeared, such as flattened, fringed and double. This development has increased the extent and intensity of leaf patterning by developing multiple sizes.

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Nowadays, they are available in multiple growth types from Morel Diffusion S.A.S: Micro, Mini, Midi, Maxi, and Magnum. More recently, the focus was extended to re-introducing fragrance, which was lost in the quest for obtaining larger plants.

Germination and seedling survival are strongly correlated to environmental factors, such as temperature and precipitation. As a consequence, pigment responsible genes may influence physiological traits linked to drought and heat stress resistance (5, 6). Bioactive metabolites are produced by the response of plants to stress.

Plants respond to stress by producing a broad spectrum of bioactive specialized metabolites. Elicitors, such as jasmonates, trigger a complex signaling circuit leading to the activation of distinct metabolic pathways, even though, the transcription factors (TFs) involved in many metabolic pathways remain unknown. Two homologous jasmonate-inducible TFs of the basic helix-loop-helix (bHLH) family, namely TRITERPENE SAPONIN BIOSYNTHESIS ACTIVATING REGULATOR1 (TSAR1) and TSAR2, which direct triterpene saponin biosynthesis in *Medicago truncatula* Gaertu and *Nicotiana tabacum* L.*,* have been reported (7-9). Also, in *Arabidopsis*, a different TF (i.e. MYC2) is responsible for the binding of sesquiterpene synthase gene promoters. This leads to an elevated release of volatile sesquiterpenes (10), proving to be an important aspect due to limited available data regarding TFs implicated in the activation of specialized terpene metabolite production.

In the case of *Cyclamen* species, habitat fragmentation, environmental degradation and tuber over-exploitation have exerted pressure on the native populations of these valuable ornamental species. Therefore, the entire genus *Cyclamen* is included in the Convention on International Trade in

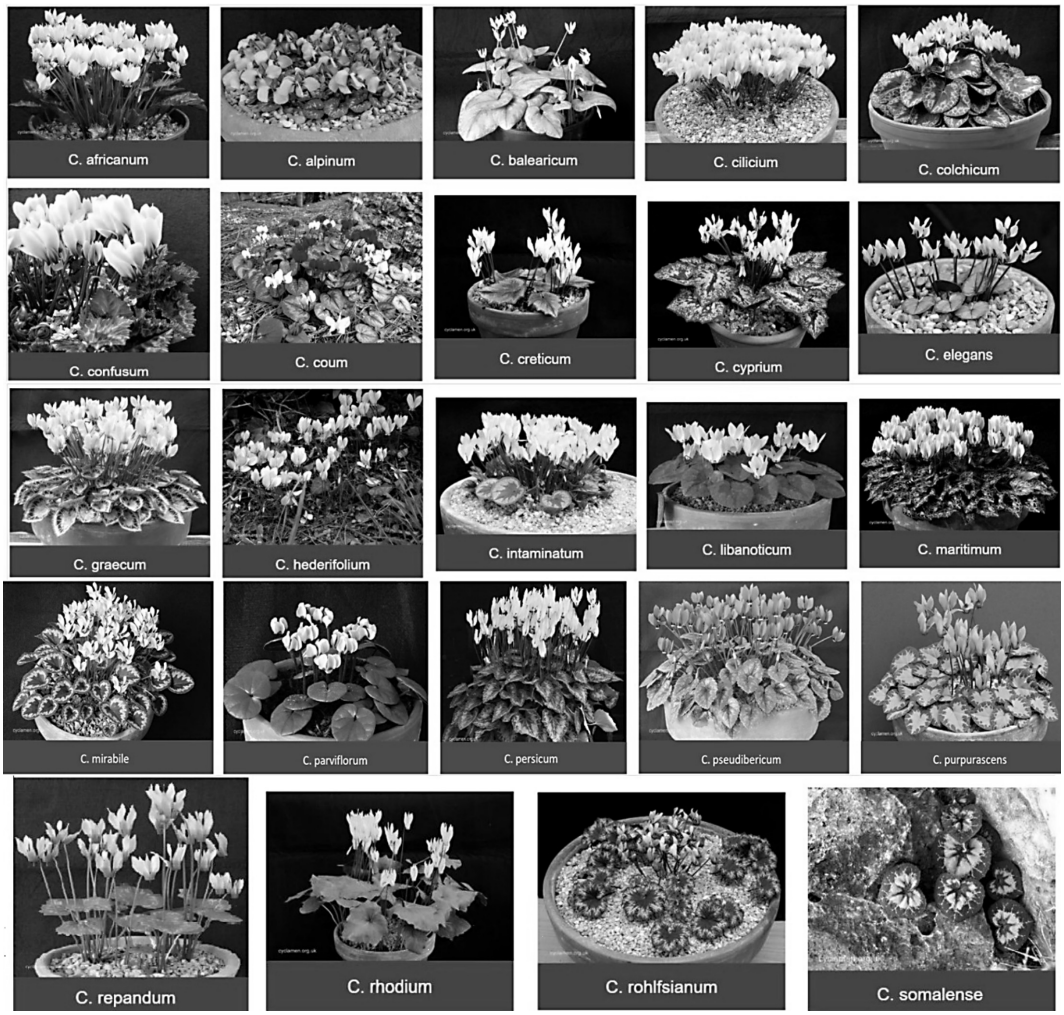

Figure 1. Genus *Cyclamen* known species (4).

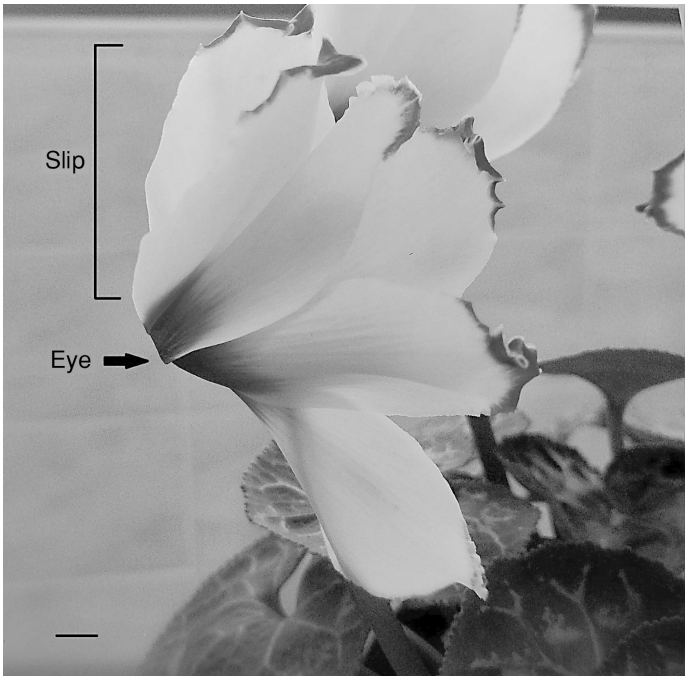

Figure 2(a). Flower characteristics of *C. persicum* cv. Victoria. Bar = 2 cm; Expression patterns of cyclamen MADS-box genes in various tissues.

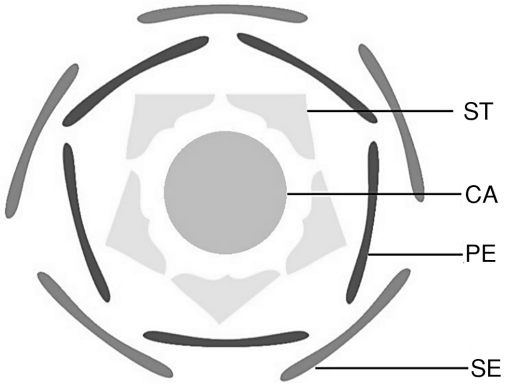

Figure 2(b). Flower characteristics of *C. persicum* cv. Victoria. Scheme of floral diagram showing the relative placement of floral organs. Lines and characters show each tissue. (SE) Sepals, (PE) Petals, (ST) Stamens, (CA) Carpel.

Endangered Species of Wild Fauna and Flora (CITES), while *C. purpurascens* Mill. is included in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (11), an important aspect due to the species significant characteristic, the fragrance.

The major pigments produced in *Cyclamen* flowers comprise flavonoids, carotenoids, chlorophylls, and betalains. Among flavonoids, anthocyanins range from red to purple and flavonol glycosides (quercetin and kaempferol glycosides) range from colorless or pale compounds. Anthocyanins and flavonol glycosides are widely distributed in many organs, especially in cyclamen flowers. Anthocyanin colors change with the presence of flavonol glycosides as co-pigments, resulting in a broad range of colors (12, 13). Also, it is well known that these above-mentioned molecules saponins, glycosides, tannins, resins, gums possess significant healing properties (14, 15).

The flowers of *C. purpurascens* Mill. and most *C. persicum* Mill. cultivars accumulate quercetin and kaempferol glycosides as major flavonols (16). In yellow-flowered *Cyclamen,* an interesting feature

in color expression and pigmentation is observed, namely isosalipurposide (ISPP), which is the main pigment in petals but is also found in some vegetative organs (17). The pollen grains of *C. pseudibericum* Hildebr are radially symmetric, isopolar, tricolporate, spheroidal and circular in polar view. Their shape is comparatively similar to *C. africanum* Boiss & Reut., *C. mirabile* Hildebr, *C. neapolitanum* and *C. purpurascens*. Furthermore, the aperture type of *C. pseudibericum* Hildebr pollen is similar to *C. africanum* Boiss & Reut., *C. hederifolium* Aiton. and *C. purpurascens* Mill., but different from *C. mirabile* Hildebr, due to the fact that *C. mirabile* pollen is tetracolporate, while *C. pseudibericum* Hildebr pollen is tricolporate (18).

Floral features are particularly important in terms of production. The size of the petals and shape of the commercial plants vary from one species to another. Today, different genotypes are available with white, purple, red-carmine or yellow petals. Although diploid and tetraploid cultivars are available, the consumers show a preference for diploid F1 cultivars (2).

Several species have interesting horticultural features, such as *C. purpurascens* Mill., which emanates a powerful scent. While it is difficult to produce interspecific hybrid seeds in most of the crosses, hybrids can result from crosses between commercial *C. persicum* Mill. and several wild-types, through *in vitro* culture (19-24).

Wild *C. purpurascens* Mill.. has small flowers with a purple slip and a deep purple eye, but *C. purpurascens* f. *album* Grey-Wilson, with a white slip and a white eye, is occasionally found in the wild. Neither form has been developed into commercial cultivars (2). However, their flowers emanate a sweet fragrance resembling rose, hyacinth or lily of the valley (25).

The biosynthesis of flower pigmentation proves to be an efficient system in elucidating gene expression and cell metabolism (3). Developing research into *Cyclamens* features and breeding techniques underline great reproduction possibilities, in addition to petal color and fragrance.

Transcription factors (TFs) as key roles in regulating the expression of biosynthesis pathway genes

Worldwide, *Cyclamen* is an important ornamental pot-flower. Various traits using molecular techniques in promoting breeding have been studied. They are commonly grown as pot flowers and recently produced as cut flowers. Hence, novel cultivars with distinctive flower morphologies, such as double flowers and fringed petals are desired by consumers and growers.

Wild *C. Persicum* Mill. has small flowers consisting of a white, pink or purple slip, which is the region excluding the eye, and a deep purple eye, meaning the region of the petal base. To better comprehend the significant features in *Cyclamen* species, for example, in *C. persicum* cv. Fuji Dark Violet, the flowers consist of a deep purple "eye" (the region of the petal base) and a purple "slip" (the region excluding the eye) with white edges (Fig. 2).

Cyclamen flowers, like those of many flowering plants, consist of four whorls. In the outermost whorl, there is a calyx consisting of five sepals. In whorl 2, there is a corolla consisting of five petals, with five stamens attached by short filaments in whorl 3. Whorl 4, in the center of the flower, contains one pistil with five fused carpels.

Modifications in flower shape, such as semi-double, double flowers and in distinct parts, like petals, sepals, give uniqueness, besides higher commercial value. In model plants, such as *Arabidopsis thaliana* (L.) Heynh. and *Antirrhinum majus* L., key TFs are responsible for identifying distinctive flower forms (26, 27).

TFs are proteins that control the rate of genetic information transcription from DNA mRNA by binding to a specific DNA sequence (28).

TFs possessing a DNA-binding region, known as MADS domain, are encoded by MADS-box family genes. These family genes involved in floral organ identity have been isolated from wide-ranging assortments of flowering plants. The advances in plant molecular genetics revealed that the mechanisms responsible for flower development have been highly conserved during evolution (29).

The function of MADS-domain proteins has been studied broadly in multiple organisms with the discovery that these proteins are involved in different plant developmental stages (30), in muscle development, neural signal transmission and tumor incidence in humans (31), cell survival and osmotic stress response in the yeast stationary phase (32).

Hindrance of conventional breeding is overcome by genetic engineering which can introduce novel traits in plants, such as flower color, fragrance, disease resistance, abiotic stress resistance, pest resistance, modification of flowering time and shape of flowers or other vegetative organs, was successfully accomplished in various genera, such as *Chrysanthemum*, *Cyclamen*, *Petunia* or *Torenia*, using diverse techniques, for instance, gene silencing (RNAi) and Chimeric REpressor gene-Silencing Technology (CRES-T) (33).

Castelán-Muńoz et al., (2019) review how MADS-domain TFs play a distinct role in abiotic stress responses during seed development, vegetative growth, flowering, and root development. Among these abiotic factors, drought, salinity, low and high temperatures, as well as nutritional deficiencies greatly affect the plants' developmental stages (35, 36).

CRES-T is another important instrument that has lately been developed as a useful analysis method of plant TFs and for plant trait genetic manipulations. For CRES-T, a TF is converted into a strong repressor by an SRDX repression domain fusion, which afterward induces a loss-of-function phenotype.

The chimeric repressor dominantly suppresses the expression of target genes, even in the presence of redundant endogenous TFs, followed by TF loss-of-function phenotype (37). CRES-T has been successfully utilized in modifying the shape of cyclamen (33), torenia (38), rose (39) and chrysanthemum (40).

Unique flower forms, such as double flower, have higher ornamental value than single-flowered phenotypes. Double flowers are characterized by several petal whorls, either by conversion of another floral organ into petals or excessive development. Aesthetic traits have long played significant importance, especially the development of double flowers in existing varieties (41, 42). Generally, the majority of double-flowered varieties were derived from single-flowered wild ancestors (43).

The induction of double-petal cyclamen flowers by introducing 35S:CpAG1-SRDX and 35S:CpAG2-SRDX into five petal cultivars has been achieved (38). Two transgenic cyclamen lines expressing 35S:CpAG1-SRDX produced double-petal flowers, instead of stamens. Introducing of 35S:CpAG2-SRDX into a cultivar which lacks CpAG1 expression led to inducing of multi-petal flowers by conversion of stamens and carpel. Also, transgenic cyclamen lines with multi-petal flowers that harbor 35S:CpAG1-SRDX and 35S:CpAG2-SRDX in a five petal cultivar, were produced. Therefore, rose-like flowers can be obtained by the expression of two chimeric AG repressors in cyclamen WT. Also, even though CpAG1 and CpAG2 have similar protein functions their roles in whorl 3 and whorl 4 remain distinctive.

Mutations occur in homeotic genes that encode TFs which control the development of distinct parts identity and exhibit the conversion of one body part into another. These mutations have been observed in *A. thaliana* (L.) Heynh. in determining not only flowering transition but also meristem specification, seed, root and fruit ripening, respectively. Their function has been widely studied and summarized in several outstanding reviews (39-45). The development of homeotic mutant flowers is made by mutation of ABCDE genes (27, 52) (Fig. 3).

Based on floral organ modifications from the quartet models (27, 53), A-class genes such as APETALA1, in *Arabidopsis*, and E-class genes are responsible for the development of sepals in the first floral whorl. B-class genes such as APETALA3 and PISTILLATA, along with A and E-class genes lead to petal formation in the second whorl. B, C and E class genes, such as AG, merge in function to specify stamens in whorl 3. C and E-class genes singularly specify carpel identities in the fourth whorl. Coming along, D-class function genes are required for ovule development. Cloning of ABCDE homeotic genes in *Arabidopsis* revealed the encoding of MADS-box TFs (38). Therefore, transformation of one organ type into another is achievable via homeotic genes.

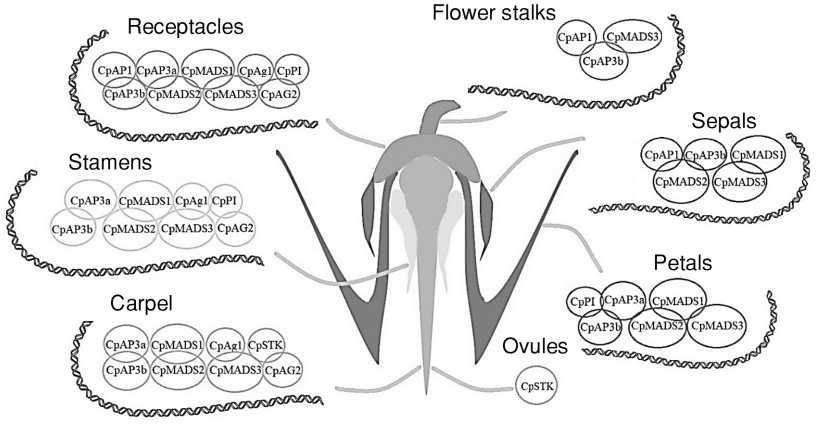

Figure 3. ABCDE model in *Cyclamen* (frontal view) and genes expression patterns in various tissues.

In *Arabidopsis*, A-class gene is AP1 required for floral meristem identity, B-class genes are AP3 and PISTILLATA (P1) and C-class gene is AG. The C-class gene is required for determining the floral meristem, as well as male and female organogenesis. The D-class genes are SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHP2 and E-class genes are represented by SEPALLATA1 (SEP1), SEP2, SEP3, and SEP4. Regarding the interaction of D and E-class proteins is they specify ovule identity and E-class genes appear to be redundant when it comes to determining the identity of sepals, petals, stamens, and carpels. For instance, the flowers of *Arabidopsis ag* mutants have no stamens or carpels, therefore losing the ability to terminate meristematic activity, resulting in a frequent structure of sepal with tens of petals (54-61, 38).

In *Cyclamen*, the single A-class gene, CpAP1 is expressed in sepals, receptacles, flower stalks and slightly in petals. The B-class genes: CpPI is expressed in petals, stamen and receptacles, CpAP3a is expressed in petals, stamen, carpels and receptacles, and CpAP3b is expressed in sepals, petals, stamen, carpels, receptacles and flower stalks. From the C-class AGAMOUS the CpAG1 and Cp AG2 genes are expressed in stamen, carpels and receptacles. The D-class gene is represented by CpSTK which has the same expression pattern as in *Arabidopsis*, namely carpels. The E-class genes in *Arabidopsis* are expressed in sepals, petals, carpel and stamen, whereas in cyclamen along with expression patterns from *Arabidopsi*s, CpMADS1 is also expressed in receptacle and flower stalks and CpMADS2 in receptacle. The CpMADS3 gene encodes a >50% identical protein to FLOWERING LOCUS C (FLC), which is expressed in all tissues in cyclamen, compared to *Arabidopsis* where it was regarded as a flowering repressor because it is not expressed in inflorescence tissues (Table 1).

Multiple interspecific hybrids, allodiploids (AB) and allotetraploids (AABB) have been obtained, which release monoterpene alcohols, sesquiterpene alcohols, phenylpropanoids/benzenoids, and the fragrance rose oxide (monoterpene) with a similar scent to *C. purpurascens* Mill. (25). Nowadays, breeders are trying to introduce fragrance in other varieties, but with few positive results, due to cross-incompatibility. The increasing demand for scented cyclamen cultivars led to the creation of scented yellow cyclamen. The crossing of allotetraploid from *C. persicum* "Golden Boy" (AA) × *C. purpurascens* (BB) resulted in pink cultivars with volatiles and perfumes similar to *C. purpurascens* Mill.

This proves to be an efficient transformation, due to the increasing demand in varieties with highly ornamental traits, such as color or perfume, especially because *C. purpurascens* Mill. represents the original fragrant specie, being the most scented compared to the other species of the genus.

The main purpose of flower pigmentation is attracting pollinators, meaning that flower color patterns are easily identifiable against their background. However, flower color is not perceived equally by all pollinators. For example, hummingbirds distinguish red flowers, but to bees, they are colorless (62). It can be foreseen that mutational inactivation of several target genes can efficiently

Table 1. TFs in Cyclamen and Arabidopsis.

Class genes	Genes		Expression pattern		Amino acid sequence sharing to *Arabidopsis*
	Cyclamen	*Arabidopsis*	*Cyclamen*	*Arabidopsis*	
A	CpAP1	AP1	SE, R, FS, mP	FS, SE	60%
B	CpPI	PI	P, ST, R	P, ST	-
	CpAP3a	AP3	P, ST, C, R	P, ST	48%
	CpAP3b	AP3	SE, P, ST, C, R, FS	P, ST	45%
C	CpAG1	AG	ST, C, R	ST, C, R	71%
	CpAG2	AG	ST, C, R	ST, C, R	68%
D	CpSTK	STK	C	C	60%
E	CpMADS1	SEP2	SE, P, ST, C, R, FS	P, ST, C	66%
	CpMADS2	SEP3	SE, P, ST, C, R,	P, ST, C	72%
Other	CpMADS3	FLC	SE, P, ST, C, R, FS, L, LS	NE	-
	CpACT	ACT	SE, P, ST, C, R, FS, L, LS	NE	-

Note: SE, Sepal; P, Petal; ST, Stamen; C, Carpel; R, Receptacle; FS, Flower Stalk; L, Leaf; LS, Leaf Stem, NE, not expressed

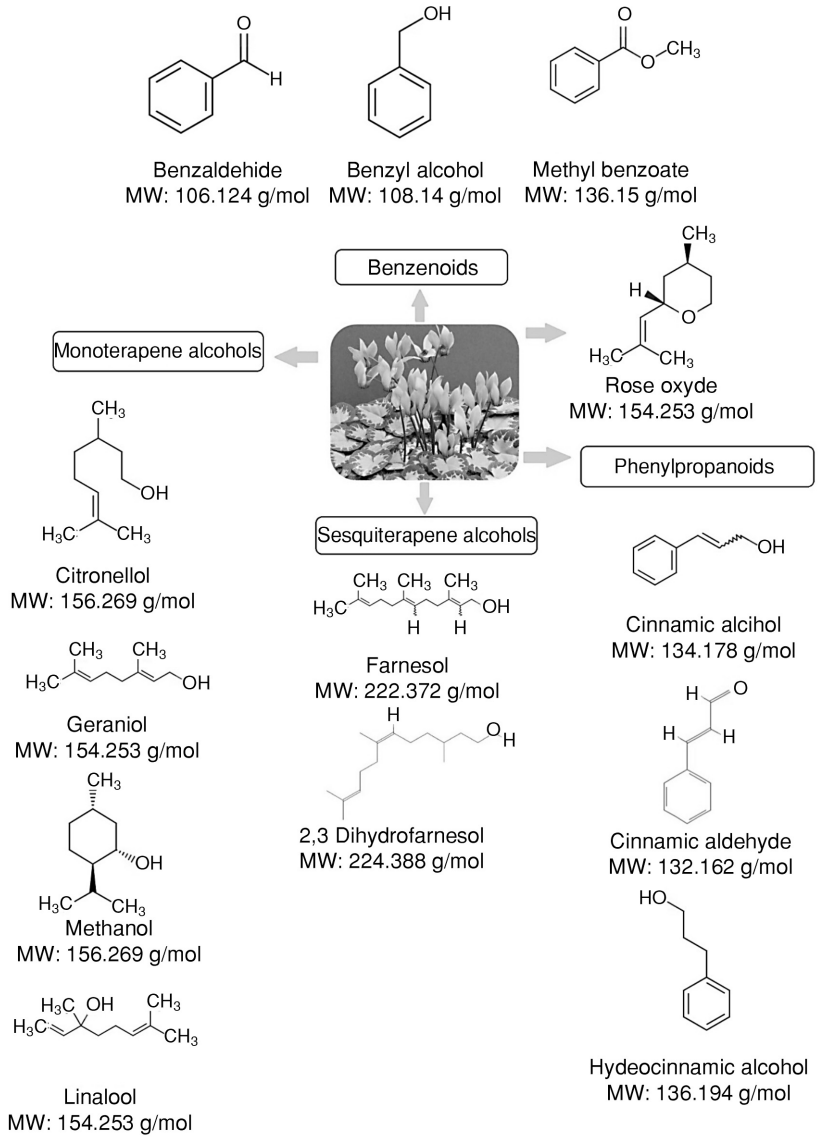

Figure 4. Volatile compounds in the flowers of *C. purpurascens* Mill.

change and ultimately predict the type of pollinators, with consequences in speciation and genetic isolation (63).

For instance, bumblebee's preference for color can be influenced by floral scent (64). A hint in nectar quality is given by flower pigmentation (65), and pollinator choices are affected by nectar rewards, respectively (66, 67). The scent has not been regarded as an important breeding characteristic due to the fact that most of the *C. persicum* Mill. cultivars release a powdery or weak woody scent. Recently it became a field of interest due to the presence of sesquiterpene hydrocarbons in *C. purpurascens* Mill. which releases a sweet fragrance resembling hyacinth, rose or lily of the valley (25) (Fig. 4). The flowers of *C. purpurascens* Mill. emit monoterpene alcohols, sesquiterpene alcohols, phenylpropanoids, benzenoids, and rose oxide. Among aromatic compounds, phenylpropanoids are major volatiles and benzenoids are minor volatiles (25, 68). Most of these sesquiterpene alcohols, such as farnesol, have been reported to exhibit anti-cancer and anti-inflammatory effects, and also alleviate allergic asthma, gliosis and edema (69). These can lead to the discovery of new natural drugs with potential uses as anticancer agents.

The major breeding goals are the creation of novel flower forms in ornamentals, increasingly defining the commercial value by its unique asset.

Floral organs are determined by five classes of homeotic genes, namely A, B, C, D, and E, whereas developing new varieties comes with the modification of these genes. Therefore, chimeric repressor constructs may save time in breeding novel varieties with double- and multi-petals, based on already existing cultivars with valuable traits such as perfume, eye-catching colors and ruffled petals. Usage of these TFs and other multiple strategies like hybridization, genetic engineering, mutation, and polyploidy, results in the possibility of developing unique flower varieties.

The examination of how such paralogous TFs exert their distinct functions, probably with slight different binding-site sequence peculiarities, will prove to be an attractive topic for future research.

General medicinal importance and pharmacognosy

Many plant extracts represent a source of secondary metabolites with different biological and pharmacological properties and play a therapeutic role in the human organism (70). The medicinal use of *Cyclamen* is known since antiquity by Egyptians, Greeks and Romans.

Investigating the chemical composition of several *Cyclamen* species, various authors have reported the presence of triterpene saponins and glycosides (71), phenolic and polyphenolic components (15) (anthocyanins, flavonoids (72)), a piperidine alkaloid and sterols (73).

Saponins (from the Latin word "*sapo*", or soap), are a class of high molecular weight secondary metabolites, characterized by a carbon skeleton derived from a 30-carbon 2,3-oxidosqualene precursor. They can be classified in steroidal (C_{27}) or triterpenoid (C_{30}) saponins, based on their carbon nucleus (aglycone). Sugar residues are linked to the aglycone, conferring an amphiphilic nature to these molecules, which is significant for their biological activities of these glycosides (74). They comprise a large variety of molecules that have multiple potential applications in pharmacology, for instance, anti-inflammatory, antibacterial, immunoadjuvant, antiplatelet, antitumoral, hypocholesterolemic, anti-HIV, insecticide, fungicide, anti-leishmanial bioactivity (75), as well as in food and cosmetics (76).

Even though they are considered to be toxic, the leaves of *C. persicum* Mill. are used as food in Palestine and other Middle East countries to make Za'amatoot. The leaves are filled with rice and meat, made into rolls before cooked and eaten with yogurt (77).

Saponins and saponin-rich extracts are used in the food industry as foaming agents for beverages, as detergents. They are known to cause hemolysis, by complexing plasma membrane sterols and by increasing membrane permeability. This asset of affecting membrane integrity is partially related to their antimycotic and antimicrobial activities. In plants, saponin functions are usually associated with resistance against pathogens, mostly fungi (78).

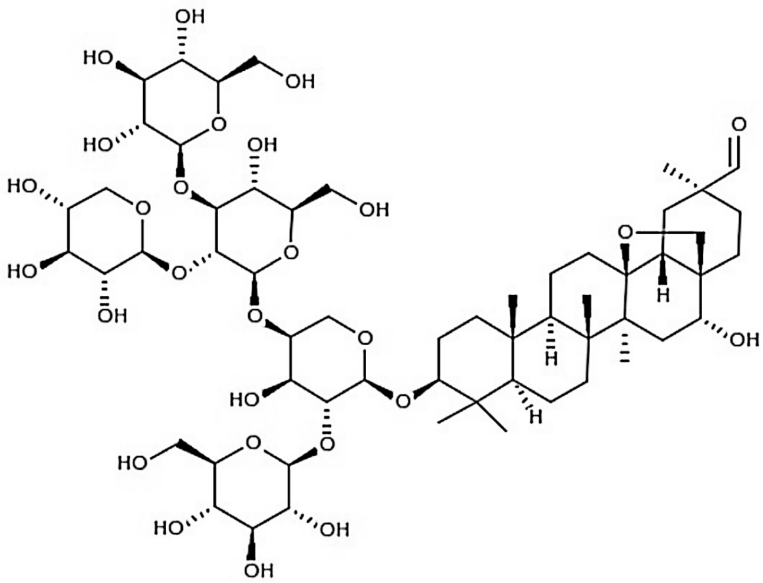

Figure 5. The chemical structure of cyclamin (toxic compound).

Table 2. Bioactivities study in various *Cyclamen spp*. extracts and respective results.

Species	Plant part	Extract	Effects	Bioactive dose	Bioactive pathway	References
C. alpinum Dammann ex Spreng	T	Ethanol extracts	Larvicidal activity against *Culex pipiens* L.	72 h at the doses of 750 and 1000 ppm	≥80.0% mortality on first and second larval stages	(82)
C. alpinum Dammann ex Spreng	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↓cytotoxic activity in the leaf extract LC_{50}: 122.56 g/ml ↑ cytotoxic activity in tuber extract LC50:9.89 g/ml	(83)
C. alpinum Dammann ex Spreng	T and L	ethanol, methanol and acetone extracts	Biolarvicidal activity against *C. pipiens* and *A. salina* Tubifex tubifex	24-, 48-, 72-h exposure of *A. salina*, *C. pipiens* at concentrations of 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1 mg/mL; 2-, 4-, 6-min of exposure of T. tubifex of ethanol extract at (1 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL and 40 mg/mL concentrations	LC_{50} (*C. pipiens*) - 0.534 mg/mL for leaf extract and 0.151 mg/mL for tuber extract after 72 h ↑ toxic LC_{50} (*T. tubifex*): 0.52 mg/mL in tuber extract and 1.32 mg/mL in leaf extract ↑ cytotoxicity LC_{50} (*A. salina*): 0.257 mg/mL for tuber extract	(84)
C. cilicium Boiss. & Heldr.	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↑ cytotoxic activity in leaf extract LC_{50}: 21.65 g/ml ↑ cytotoxic activity in tuber extract LC_{50}: 19.31 g/ml	(83)
C. coum Mill.	T	*n*-butanolic *C. coum* extract-ciprofloxacin combination	Cytotoxic effect against HT-29 and *P. aeruginosa* (ATCC 27853) biofilms	*n*-butanolic extract from 25 to 150 µg/mL added to 96-well microtiter plate after 48 h and 72 h incubation	↑ cytotoxicity against 1-and 3-day-old *P. aeruginosa* PAO1 biofilms ↓ apoptosis in HT-29 by MTT test	(85)
C. coum Mill.	T	*n*-butanolic extract - ciprofloxacin combination	Eliminate 1- and 3-day-old *P. aeruginosa* PA01 and *P. aeruginosa* ATCC 8821M biofilms	MIC (µg/mL) n-butanolic extract *P. aeruginosa* Strains: 254-257 MIC (µg/mL) Ciprofloxacin: *P. aeruginosa* Strains: 0.25-8 MBC (µg/mL) Ciprofloxacin: *P. aeruginosa* Strains: 0.5-16	↑ apoptosis	(86)
C. coum Mill.	T	Aglycon and glycosidic saponin extracts	Anticandida activity against *C. albicans* (ATCC 10231 and 3 isolates), *C. tropicalis* (ATCC 0750 and 6 isolates) and *C. krusei* (1 isolate)	20 µg/ml of *n*-butanolic extract against *C. albicans* and *C. tropicalis*; 10 µg/mL of aglycone aqueous extract against *C. tropicalis* and *C. krusei*	↑ cytotoxicity of aglyconic aqueous phase of the extract ↔ cytotoxicity of glycosidic and aglyconic aqueous extracts on C. albicans and C. tropicalis	(87)
C. coum Mill.	T	*n*-butanolic extract -ciprofloxacin combination	Decrease in the expression of protein CTC, superoxide dismutase, GroEL, and Hcp1	MIC (µg/mL) n-butanolic extract *P. Aeruginosa* PAO1 - 256 MIC (µg/mL) ciprofloxacin *P. Aeruginosa* PAO1 - 0.25 µg/mL	↑ apoptosis PAO1 ↑ lung protection	(88)

Table 2. Continued.

Species	Plant part	Extract	Effects	Bioactive dose	Bioactive pathway	References
C. coum Mill.	L and P	Methanolic extract	Antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and Methicillin-Resistant *S. aureus* (MRSA)	Antioxidant activity IC_{50}: 31 µg/mL plant extract MIC: 6.25-12.5 mg/mL	↓ antibacterial activity Total flavonoids, phenols, and tannins: 60.88 mg QUE/g, 32.7 mg GAE/g and 11.7 mg CAE/g	(89)
C. coum subsp. *coum*	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↓ cytotoxic activity in the leaf extract LC50: 84.88 g/ml ↑ cytotoxic activity in tuber extract LC50:25.87 g/ml	(83)
C. europaeum L.	T	triterpenoid saponins	317 patients with chronic rhinosinusitis divided in 3 groups: group 1, N = 128, group 2, N = 90, group 3, N = 99	group 1: oral antibiotic + intranasal CE (2.6 mg Sinuforte Nasal spray); group 2: intranasal CE; group 3: oral antibiotic, administered daily for 8 days	↓ recurrence ↑ symptom relief	(94)
C. europaeum L.	T	lyophilized extract	29 patients divided in 2 groups: G1 (placebo)= 16 G2(CE) =13	G1: sterile water G2: 1.3 mg lyophilized CE extract with a once-daily treatment for 7 days	G1: ↓ headache, ear pain, gastritis, back pain, conjunctival hemorrhage G2: ↔ rhinalgia, sneezing, throat irritation ↑ sinus opacification ↓ adverse effects	(95)
C. europaeum L.	T	lyophilized extract	99 participants divided in 2 groups: G1 (placebo) = 51; G2 (CE) = 48	G1= 500 mg of amoxicillin three times daily for 8 days G2: 1.3 mg lyophilized CE extract for 15 days	G1:↔-epistaxis ↓nasal irritation and vertigo G2: ↓ facial pain ↓ in endoscopically-assessed mucosal obstruction ↔ nasal burning, epistaxis ↓ adverse effects	(96)
C. graecum Link	T and L	methanol, ethanol, acetone and petroleum benzene extract	Antioxidant activity evaluated by using DPPH and β-carotene-linoleic acid assays	Free scavenging activity (97.3 ± 0.55%) of CLE and antioxidant activity (80.2 ± 0.60%) of CLE, extracted with 1 mg/mL concentration	↑ free scavenging activity of CLE and ↓ of CLA ↑ antioxidant activity of CLE and ↓ of CLA	(90)
C. graecum Link	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↑ cytotoxic activity in the leaf extract LC_{50}: 4.20 g/ml ↑ cytotoxic activity in tuber extract LC_{50}:2.93 g/ml	(83)
C. hederifolium Aiton	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↓ cytotoxic activity in the leaf extract LC_{50}: 85.95 g/ml ↓ cytotoxic activity in tuber extract LC_{50}:72.34 g/ml	(83)

Table 2. Continued.

Species	Plant part	Extract	Effects	Bioactive dose	Bioactive pathway	References
C. mirabile Hildebr	T	ethanol extracts	Larvicidal activity against *Culex pipiens* L.	72 h at the doses of 450 and 500 ppm	≥90% mortality in first and second stages	(82)
C. mirabile Hildebr	T	Ethanol extract	Mastitis	Antibacterial activity of ethanol extract (1625 µg/mL). against Coagulase-negative *staphylococci*-36 (CNS-36)	↑ antibacterial on CNS-36 and ↓ on CNS-37 ↑ antioxidant activity	(91)
C. mirabile Hildebr	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salin*a L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↓ cytotoxic activity in the leaf extract LC_{50}: 89.02 g/ml ↑ cytotoxic activity in tuber extract LC_{50}:25.88 g/ml	(83)
C. parviflorum Pobed.	T and L	ethanol extracts	Significant biolarvacidal activity against *C. pipiens* and insignificant effect against *M. domestica*	Inhalation LC50 - 173.33 ppm and LC90 - 291.50 ppm	↔ toxic	(92)
C. persicum Mill.	T	acetone, ethanol and methanol extracts	*S. pyogenes, S. aureus, E. faecalis, P. mirabilis, K. pneumoniae, E. cloacae, P. aeruginosa, S. flexneri* and *Candida* species	(50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ mL)	↑ activity of ethanol extract against *S. pyogenes* (12.5 mg/mL) ↔ activity of acetone, ethanol and methanol extract ↓ MBC: 50 mg/mL on gram-positive bacteria ↑ activity against gram-negative bacteria ↑ activity against Candida species	(93)
C. persicum Mill.	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↑ cytotoxic activity in the leaf extract LC_{50}: 23.04 g/ml ↑ cytotoxic activity in tuber extract LC_{50}:3.60 g/ml	(83)
C. persicum Mill.	T	Methanol, ethanol, hexane; and water extract	gram-positive bacteria (*S. aureus* and *B. subtilis*); gram-negative (*P. aeruginosa* and *E. coli)*	2 to 10 h at a dose of 50µl of each extract	↑ antibacterial activity against *S. aureus* and *B. subtilis* ↓ activity against *P. aeruginosa* and *E. coli*	(97)
C. pseudibericum Hildebr.	T and L	Ethanol extracts	Cytotoxicity against brine shrimp (*Artemia salina* L.) lethality test	24 h at doses of 10, 50, 500 and 1000 g/ml	↑ cytotoxic activity in the leaf extract LC_{50}: 533.22 g/ml ↓ cytotoxic activity in tuber extract LC_{50}: 206.07 g/ml	(83)

Note: AB - Antibacterial activity; AF - Antifungal activity; ATCC - American Type Culture Collection; CE - C. europaeum plant extract; CLE - Leaf-Ethanol extract; CLA - Leaf-Aceton extract; MBC - Minimum bactericidal concentrations; MFC - Minimum fungicidal concentrations; MIC - Minimum inhibitory concentrations; L - Leaves; P-Petioles; T - Tubers, ↑- Increase; ↔ Moderate; ↓-Decrease

Scientific data suggests that the risk of chronic diseases could be significantly reduced by antioxidants. This includes cancer, diabetes mellitus and heart disease. According to folk medicine several *Cyclamen* species were used for their therapeutic properties and early investigation resulted in the isolation of triterpenic saponins (70).

Çaliş et al., (1997b) isolated four saponins cyclaminorin, deglucocyclamin, cyclacoumin and mirabilin lactone from the tubers of *C. coum* Mill., which are used in Turkish folk medicine against infertility (71). In the same year, along with the saponins isolated from *C. coum* Mill., new compounds were found in *C. mirabile* Hildebr.: cyclamin, isocyclamin and mirabilin. They possess antibacterial and antifungal activities (79) (Table 2). Also, new triterpenoid saponins, coumoside A and coumoside B, have been isolated from the whole plant of *C. coum* Mill. (73, 80).

All cyclamen plants are dangerous due to the toxic saponin known as cyclamin, contained in their fresh bulb, which is a white opaque substance that can absorb up to 45% water and become transparent (Fig. 5). Cyclamin is soluble in alcohol and turns brown on exposure to light. When dissolved in water, it produces foam by frothing test and by heating has the unique property of coagulation. Concentrated sulfuric acid colors cyclamin in purple red, which disappears with water addition.

Cyclamin is an irritant compound that causes gastroenteritis, bloody stools, dizziness, seizures and even death by asphyxiation. Studied by many physiologists, cyclamin was viewed merely as a local irritant (81).

Homeopathic essence of fresh tubers harvested in spring has shown potential for use against a variety of ailments, such as: headaches, common cold, digestive disorders, flatulence, colic attacks, chlorosis due to amenorrhea, rheumatism and gout without febrile events, cardiac neurosis, dysmenorrhea, worms, cramps and various skin diseases (81). Therefore, interactions with medical drugs should be avoided.

In old times, *Cyclamen elegans* Boiss. was used as a remedy in muscle spasms, headaches, and toothaches. Other medicinal plants, such as *Atropa caucasica* Kreyer. and *Rosa damascena* Mill.. were used as a treatment for these conditions. Also, *C. elegans* was used in spleen diseases, treatment replaced with *Cucurbita pepo* L. and *Rumex confertus* Wild. (98). However, their toxicity should be considered when other safe treatments are available. People all over the world deal with the toxic compounds (xenobiotics) of their region and the detoxication mechanisms were adapted over generations to optimize the process. This was implying adaptations of the organism that include mutations, polymorphisms so that the detoxification process for each ethnicity in each region became more effective for its xenobiotics.

Thus, each ethnic group can consume with reasonable security the products of their geographic area, previously selected over generations, but the same is false when they cross ethnicities and consume products of different origins. Depending on the time point when a mutation occurred in the history of mankind, particular mutations may be unique for a certain population or show differences in their frequency when comparing ethnicities, leading to population-specific responses to drugs and other xenobiotics (100).

From what has been explained in the previous paragraph, the consumption recommendation of medicinal plants outside their area of origin for other ethnicities should be done with extreme caution. Despite all that we have just mentioned, the development of new drugs is absolutely transverse and has all these issues under consideration. Bacteria are increasingly resistant to antibiotics and the development of new drugs is very important. For *Cyclamen mirabile* Hildebr and *Cyclamen trochopteranthum* O. Schwarz. the antimicrobial activity was verified against different microorganisms: gram-negative (*Escherichia coli*), gram-positive (*Bacillus subtilis*) and fungus (*Candida albicans*). They have low antimicrobial activity but have significant antifungal activity (101).

Plant-based medicines prove to have an efficient role in the treatment of biofilm diseases and also have fewer side effects compared to synthetic medicines (102, 103). *Cyclamen* species are also well known for their medicinal properties and application in pharmaceutical industry.

Infections caused by *Pseudomonas aeruginosa* biofilm are the major causes of death in patients with cystic fibrosis (CF). The bacterial pathogen *P. aeruginosa* is one of the most significant agents involved in chronic lung infections in CF patients. Biofilm cells are known to be 1000 times more resistant to antibiotics compared to motile cells, allowing them to recolonize the host organs despite a series of antibiotic therapy.

C. coum Mill. extract revealed antibacterial activity against the growth of planktonic *P. aeruginosa* strains. The results showed that the combination of *n*-butanolic *C. coum* extract and ciprofloxacin inhibited *P. aeruginosa* biofilm development. Also, the *n*-butanolic *C. coum* extract showed an

insignificant cytotoxic effect against HT-29 after 48 hours and 72 hours incubation (85).

The tubers of *Cyclamen* species are also known for their larvicidal activity. Therefore, the larvicidal activity of the tuber extracts isolated from two species of *Cyclamen* (*C. mirabile* Hildebr. and *C. alpinum* Dammann ex. Sprenger) against *Culex pipiens* L. (Diptera: Culicidae) was evaluated (82). Their results showed larval stage mortality after 12 h exposure and that *C. mirabile* was more effective than *C. alpinum*.

Table 3. Screening for anticancer potential of extracts and isolated compounds from *Cyclamen spp*.

Cyclamen spp. used in the essay	Plant part	Type of solvent extract or bioactive substances	Cancer cell lines	Bioactivity	References
Cyclamen spp. tubers	T	Saponin alcoholic extract	Walker carcinosarcoma 256	↑ cytotoxicity	(104)
Cyclamen spp. and *Ardisia japonica*	T	Ethanolic extract of cyclamin	Bel-7402, HepG2 and HL-7702	↑ cytotoxicity in Bel7402 and HepG2; in HL-7702	(137)
C. alpinum Dammann ex Spreng.	T	Methanolic extract of yclamin, desgluco-ccyclamin I, mirabilin	HCT 116 and HT-29	↓ cytotoxicity in HCT 116 and HT-29	(138)
C. alpinum Dammann ex Spreng.	T	Ethanol extract	Subcellular fractions of rat tissues	↑ Hepatic CYP2E1, CYP1A2 protein and CYP2C6 mRNA level	(139)
C. coum Mill.	T	Cyclaminorin, deglucocyclamin, cyclacoumin, cyclamin, isocyclamin, mirabilin and mirabilin lactone	Isolated Rat Uterine horns	↑ uterocontractile activity	(79)
C. coum Mill.	MP	Methanol and water extracts	HeLa and H1299	↑ cytotoxicity in HeLa and NSCLC H1299	(140)
C. hederifolium Aiton.	T	Hederifolioside A, B, C, D and E	Hela, H-446, HT-29, U937	↓ apoptosis	(108)
C. libanoticum Hildebr.	T	Triterpene saponins: saxifragifolin B and cyclamin	SK-BR-3, HT-29, HepG2/3A, NCI-H1299, BXPC-3, 22RV1, DMEM	↓ antioxidant ↓ citotoxicity	(141)
C. persicum Mill.	T	Alcoholic extract	Human carcinoma of the nasopharyn	↑ apoptosis	(142)
C. persicum Mill.	T	Active saponin-fraction	Walker carcinosarcoma 256	↑ apoptosis	(142)
C. persicum Mill.	T	Triterpene saponins: saxifragifolin B and cyclamin	SK-BR-3, HT-29, HepG2/3A, NCI-H1299, BXPC-3, 22RV1, DMEM	↓ antioxidant ↓ cytotoxicity	(141)
C. persicum Mill.	R	Decoction (oral)	NS	↓ cytotoxicity	(143)
C. persicum Mill.	T	3,5-Dithiahexanol 5,5-dioxide, 1-(2-Nitrophenyl) piperazine	NS	↑ apoptosis	(144)
C. pseudibericum Hildebr.	T	n-butanolic saponin extract dissolved in dimethyl sulphoxide (DMSO)	A549 NSCLC cell line	IC50: 41.64±2.35µg/mL ↑ cytotocicity on A549 cells ↓ number of A549 cells	(145)
C. purpurascens Mill.	T	Cyclamin, isocyclamin and methylcyclamin	L929 fibroblastic	↑ cytotoxicity ↓ cell viability	(146)

Note: NS - Not Specified; R - Roots; T - Tubers; MP - Multiple parts; ↑- High; ↓- Low

Extracts of *Cyclamen spp*. tubers exhibited *in vitro* cytotoxic (104), spermicidal (105) and antimicrobial activities (106). It was reported to have sedative, purgative, abortive, laxative, vomiting emmenagogue and antihelmintic activity (107, 108).

A study published in 2014 evaluated the antioxidant and antibacterial activity of three crude extracts of *C. africanum* Boiss & Reut.; namely, dichloromethane (DCM), methanol extract (ME) and methanol-water (MW), was evaluated (109). The results clearly show that the crude extracts of *C. africanum* Boiss & Reut. only possess antioxidant activity, MW and DCM having a slight activity against *Pseudomonas aeruginosa*.

In a recent study, Dusen et al. (2016) evaluated the cytotoxic activity of the ethanol extracts of multiple *Cyclamen* L. taxa (*C. alpinum* Dammann ex Spreng., *C. cilicium* Boiss. & Heldr., *C. coum* Mill. subsp. *coum*, *C. graecum* Link, *C. hederifolium* Aiton, *C. mirabile* Hildebr., *C. persicum* Mill., and *C. pseudibericum* Hildebr.) by using brine shrimp (*Artemia salina* L.) lethality test and the LC_{50} values of the extracts were also determined.

The aim of a study (110) was to investigate the antibacterial activity of *C. cilicium* Boiss. & Heldr., *C. pseudibericum* Hildebr. and *C. hederifolium* Aiton ethanol tuber extract against five bacterial fish pathogens, namely, *V salmoninarum*, *S. epidermidis*, *L. garvieae*, *V. anguillarum,* and *Y. ruckeri*, as well as their radical scavenging activity potentials. Their results showed that all the extracts exhibited moderate antibacterial activity in the order: *C. cilicium*> *C. pseudibericum*>C. *hederifolium* against four bacteria with the exception of *V. anguillarum*.

Around ten years ago, a new product obtained from plant extracts (*C. europaeum*), in the form of nasal spray (Nasodren®) has been developed. It acts on mucous membranes of nasal and paranasal sinuses, activating physiological mechanisms for clearing nasal mucosa and facilitating drainage of the accumulated secretions (111, 112). An exploratory trial in patients (n = 29) with acute rhinosinusitis was conducted to evaluate the efficacy and safety of *C. europaeum* extract with the results of reduced sinus opacification compared with placebo treatment (95). In the same yea,r Pfaar et al. (2012) repeated the experiment with 99 patients. Both studies used placebo in the control group and the same dose (1.3 mg/day) of *C. europaeum* lyophilized extract. Treatment duration in the studies was 15 days in Pfaar et al. (2012) and seven days in Ponikau et al. (2012). Uncertain results were observed in the former study regarding the improvement of nasal obstruction and edema. In 2018 Barua et al. reviewed the studies above mentioned concluding that patients who received *C. europaeum* rather than a non-active substance reported more side effects like nasal irritation, sneezing, and mild nasal bleeding, but with no major side effects.

In a study conducted by Evseenko et al. (2019) the *C. europaeum* tubers extract with concentration commonly used for human rhinosinusitis treatment was tested as a mucosal adjuvant in experimental intranasal immunization of guinea pigs with concentrated commercially available influenza trivalent vaccine and subsequent infection with influenza strain A/California/04/2009 H1N1pdm. Their results showed that dual intranasal immunization with vaccine compound consisting of 7.5 μg of each hemagglutinin and 500 μg of *Cyclamen* extract in 50 μL induced reciprocal geometric mean titer (GMT) on day 21 after immunization 40 (5-640) against H1N1pdm; 43.20 (5-1280) against H3N2; 10.80 (5-80) against influenza B. The animals with hemagglutination inhibition antibody (HI) titers 1/80 against cell-derived antigen were completely protected against challenge with A/California/04/2009 H1N1pdm09.

Due to their saponin content, *Cyclamen spp*. have respiratory allergic (111, 112) and plant poisonings effects (114, 115). Also, they exert several allergenic effects (114, 116) and analgesic antiinflammatory effects (115, 117).

In general, the antioxidant activity of various solvent extracts from different parts (tuber and leaves) of *C. mirabile* Hildebr. were evaluated, using petroleum ether, acetone, methanol, and water. Also, other antioxidant properties were assessed, including free scavenging activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power and total phenolic content (15).

Also, using synthetic preparations, it is likely to obtain similar bioactivities as for pure native compounds, as well as their analogs, through controlled structural adjustments formerly devised by medicinal chemistry studies. This will allow the discovery of new drugs, nevertheless, which is a challenging strategy due to the complex chemical structure of saponins, including various substituents, asymmetric carbons, and sugar moiety diversity. For several classes of compounds, the preparation of novel saponins is now available, including *Quillaja* saponins (118, 119) steroidal saponins (120-122) and oleanolic acid derivatives (123, 124) from various crude matrix sources.

Therefore, the application in the pharmaceutical industry and as a future source of dietary macro- and microelements (125) proves to be achievable with further studies.

Anticancer potential of *Cyclamen spp.* constituents

World Health Organization gave cancer as the second leading cause of death globally (1 in 6 deaths) responsible for an estimated 9.6 million deaths in 2018. They also emphasize that approximately 70% of deaths from cancer occur in low- and middle-income countries, while around one third of deaths from cancer are due to the 5 leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use (126). In addition to these data, the Institute for Health Metrics and Evaluation (IHME), an independent global health research center at the University of Washington, states cancer as the second leading cause of death in the USA (127). Therefore, the development of preventive and therapeutic strategies is crucial to invert this tendency.

Newman and Cragg (128) review the main drugs brought to the market between 1981 and 2014 and, from the 128 new anticancer medicines, only 20 were exclusively synthetic sourced. From the chemotherapeutic drugs now available and used in therapeutic protocols against cancer, some examples can be given as the already isolated camptothecin, paclitaxel and vincristine from *Camptotheca acuminata* Decne., *Taxus brevifolia* Nutt. and *Catharanthus roseus* (L.) G. Don., respectively (129). Due to the continuing expansion of plant-derived anticancer agents that have arrived at 30%, this proves to be an important aspect in developing novel chemotherapeutics from natural sources (128-130).

The continuing development of plant-derived anticancer agents, which account 30%, leads to the expansion of natural-sourced chemotherapeutics development (128-130). Drug development programs, particularly from endemic plant resources remain significantly important in discovering novel cytotoxic agents.

Therefore, an increasing interest in the pharmacological research section leans toward new molecular targets in anticancer drug design, such as different chemical compounds (Table 3) (131). The plant kingdom proves to be a significant resource of chemical constituents with cytotoxic and antitumor activities. This is due to plant biosynthesis of an assortment of structurally varied metabolites (132, 133). For instance, diverse plant sources from Turkey, including *Cyclamen,* are prone to act as efficiently anticancer agents (134, 135).

New resources of plant active compounds have encouraged researchers to develop new anticancer drugs. Throughout time, natural products have been used in preventing and treating various diseases, including cancer, therefore proving to be useful candidates in developing new anticancer remedies (136).

Also, *in vitro* cytotoxic effect of this new commercial plant extract from *C. europaeum* was investigated in L929 culture. At lower concentrations, *in vitro* cell viability was maintained, but at higher concentrations, the extract had a potentially toxic effect on cell viability and cell morphology (146).

In a recent study conducted by Stanojević and his colleagues (2018), the bioactive compounds and mineral composition of the aqueous extract from wild cyclamen tubers (*Cyclamen purpurascens* Mill.) were assessed. The most abundant components were found to be isocyclamin and desglucocyclamin I. It should be highlighted that these saponins exert cytotoxic activity on human colorectal cancer cells (HCT 116 and HT-29) (125).

C. coum Mill. is known to be a traditional medicinal plant in Turkey. Although this plant is used for decorative purposes, its medicinal properties are yet to be explored. There is little knowledge of the anti-tumor potential of this plant (108, 147). While this plant is commonly used as strictly ornamental, its medicinal properties in solid cancer cells have remained unexplored. Its anticancer properties and the probability of the extract to induce any cytotoxicity in solid cancer cells have not been previously thoroughly investigated. Therefore, the cytotoxic effects on HeLa and NSCLC H1299 were examined, with the result that Cyclamen extract induced cell death of HeLa and H1299 cells in a dose-dependent manner.

The capacity of the *Cyclamen* extract to induce apoptosis was also analyzed with the TUNEL assay. For the first time, it was reported that *C. coum* extract, an endemic plant for Turkey, Bulgaria, Georgia, and the Middle East, can be further investigated with the possibility of finding innovative anticancer compounds (140).

It is thought that the epithelial-to-mesenchymal transition (EMT) enhances malignant tumor progress. The transcriptional repressor zinc-finger E-box binding homeobox 1 (ZEB1) is a significant inducer of EMT in different human tumors, such as breast, lung, liver, pancreatic and ovarian cancers and has recently been shown to boost invasion by tumor cells (148, 140). Also, deregulation of miRNAs affects normal cell growth and development, leading to an assortment of diseases, such as cancer and cardiovascular disease (149, 150). The levels at which miRNAs are expressed in tumor tissues differs from those expressed in normal

ones, suggesting that specific miRNAs could be used as biomarkers in evaluating disease prognosis (151).

In a different study, Karagur et al. (2018) investigated the cytotoxicity of *C. pseudibericum* Hildebr. saponin-rich tuber extract of different concentrations (1, 10, 50, 100, 250, 500, 1000 µg/mL) on the capacity of non-small cell lung cancer line A549 cells to proliferate, invade and migrate. Also, they examined the expression levels of multiple invasion–migration-related miRNAs to identify those which directly target ZEB1. Their results showed that the half-minimal (50%) inhibitory concentration dose in the A549 cells was determined to be 41.64 ± 2.35 µg/mL. The overexpression of miRNA miR-200c hindered the EMT by increasing the expression of E-cadherin and decreasing the expression of both N-cadherin and vimentin through the direct targeting of ZEB1.

These findings strongly suggest that the saponin-rich tuber extracts of multiple *Cyclamen* species may have considerable anti-cancer properties in several cancer types. Further studies are required to elucidate the molecular-based mechanism involved in the EMT process of the extracts along with the isolation and identification of active saponin components. The data are scarce but show a probable potential that could help in the design development of new cytotoxic agents using cyclamen compounds as models.

Concluding remarks

This review highlights the importance of *Cyclamen* species as ornamental pot plants and as a medicinal plant due to the content in bioactive compounds that can be used in many types of diseases. As described, the formation of organs and their development is controlled by key TFs. The ABCDE model identified in *A. thaliana* (L.) Heynh. is also responsible for flower phenotype in *Cyclamen*.

It is generally known that plants have wide-ranging medicinal properties and their secondary metabolites are used as specialty chemicals, such as drugs, insecticides, dyes, fragrances, and flavors. The focus of many studies are the extracts and isolated compounds which shown promising results in inducing apoptosis in different cell cultures, such as Walker carcinosarcoma 256, HeLa, NSCLC H1299 and cytotoxicity in Bel7402 and HepG2.

For example, the sesquiterpene hydrocarbons are very effective in both curing and protecting against numerous diseases. In addition, it was demonstrated that *C. europaeum* extract has moderate efficacy in treating chronic rhinosinusitis.

An important factor in identifying new medicine-candidate molecules involves scientific evaluation of traditional plant-based healing practices. This makes *Cyclamen* a perfect candidate to be explored for future therapeutic applications.

Abbreviations

22RV1 prostate carcinoma
AMPK 5'adenosine-phosphate activated protein kinase
ARE antioxidant response element
Bel7402 hepatocellular carcinoma cell line
BXPC-3 pancreatic carcinoma
CF cystic fibrosis
CITES Convention on International Trade in Endangered Species of Wild Fauna and Flora
CNS-36 coagulase-negative staphylococci-36
DCM dichloromethane
DMEM human fibroblasts
DPPH 2,2-diphenyl-1-picryl-hydrazyl-hydrate
ERK1/2 extracellular signal-regulated kinase
H-446 human lung cancer cells
HCT 116 human colorectal carcinoma cell line
HeLa human cervical cancer cells
HepG2 human liver cancer cells
HepG2/3A hepatocellular carcinoma
HL-7702 Normal human hepatic cell line
HT-29 human colorectal adenocarcinoma cell line
ISPP isosalipurposide
L929 mouse fibroblast culture
ME methanol extract
MW methanol-water extract
NCI-H1299 lung carcinoma
Nrf2, NF-E2-related factor
NSCLC non-small cell lung cancer lines
SK-BR-3 breast adenocarcinoma cells
t-BHP tert-butyl hydroperoxide
TFs transcription factors
U937 human leukemia cells

Conflict of interest

The authors declare no conflicts of interest.

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Received: 8.04.2019