Research Article
Curr. Pers. MAPs, (2020); 3(2): 121-127

Current Perspectives on Medicinal and Aromatic Plants

An International Journal
ISSN: 2619-9645 | e-ISSN: 2667-5722

A Study on Antimicrobial and Antioxidant Activities of *Cyclamen coum Colchicum turcicum* and *Colchicum bornmuelleri* Species

Fatih ÖZCAN, Alican Bahadır SEMERCİ, Kenan TUNÇ*

Department of Biology, Faculty of Arts and Sciences, Sakarya University, Sakarya, TURKEY
*Corresponding author : ktunc@sakarya.edu.tr

https://doi.org/10.38093/cupmap.834545

Received : 03/12/2020
Accepted : 19/12/2020

Abstract

In this study, antimicrobial effects of *Cyclamen coum* Mill., *Colchicum bornmuelleri* Freyn and *Colchicum turcicum* Janka extracts prepared with ethanol against *B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *S.typhimurium* ATCC 14028, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 1029 and *S. aureus* ATCC 29213 microorganism were investigated by using Disk Diffusion method. In additon, DPPH scavening activities, antioxidant activities and total phenolic content were determined with Folin-Ciocalteu method. It was determined that extracts obtained from *C. coum* plant showed moderate antifungal activity on *C. albicans*. It has been determined that aerial extracts of *C. bornmuelleri* and *C. turcicum* plants have antibacterial effects on *E. faecalis*. These extracts did not showed antibacterial activty on other test microorganisms.The highest and lowest antioxidant activity results (IC_{50} values) obtained from the extracts were determined as *Colchicum turcicum* aerial parts 14.2 µg/mL, *Colchicum bornmuelleri* aerial parts 768,65 µg/mL. When the extracts used in this study were compared with the standart antioxidant ascorbic acid, it was found that the aboveground extracts of *C. bornmuelleri* and *C. turcicum* showed high antioxidant activity. The total amounts of phenolic substances was determined as 191,85 mg GA/100 g in the extracts obtained from the aerial part of the *C. bornmuelleri* plant.

Key Words: Antimicrobial, Antioxidant, Geophyt, Total phenolic content

1. Introduction

Geophytes are bulbous, tuberous and rhizome plants whose stems can store nutrients by differentiating under the ground. These plants are spread around the world in regions where the Mediterranean climate prevails, with warm and humid winters and hot and dry summers (Tanrıverdi, 2019). In addition to being used as an ornamental plant with its beautiful leaves and flowers, it has also been widely used medicinally and aromatically. Medicinal aromatic plants are known to have antimicrobial and antioxidant properties due to the alkaloids, tannins, flavonoids, phenolic compounds and fatty acids they contain (İnceçayır et. al., 2019). These compounds have mechanisms of action, such as scavenging free radicals, chelating metal, reducing or inhibiting reactive oxygen generation. In addition, they prevent the formation of free radicals with the aromatic rings they contain (Çoban and Batır, 2010).

Cylamen coum, which is a geophyte plant species, has flattened spherical tubers that

are usually smaller than 3.5 cm (Davis, 1984). This plant is known with local names such as ground nut and pork apple in Anatolia (Güner, 2012). It is frequently preferred as an ornamental plant with its heart-shaped leaves and pink flowers. Apart from its use as an ornamental plant, it is also used in the pharmaceutical and chemical industry due to its secondary metabolites such as coumoside, cyclominorin, deglucocyclamine, cyclacumin, mirabilin lacton, cyclamiretin and cyclamigenin (Çalış et. al., 1997; Yaylı et. al., 1998; Bokov et. Al., 2020). Although the *Colchicum* genus is more common in the Northern Hemisphere, especially in the Mediterranean Region, it spreads to the North of Europe, North Africa and the Himalayas (Düşen, 2005). Some of the *Colchicum* species bloom in the spring, while others bloom in the fall. Fall-blooming species usually have larger bulbs and seeds than spring-blooming varieties. *Colchicum*, one of the natural geophytes of Anatolia, is frequently used both as an ornamental plant and as a medicinal plant with various alkaloids it contains. *Colchicum* species are given local names such as Bitterberry, Belladonna, Orchid Flower, Lycophor and Tarhan Flower (Düşen, 2005).

Colchicine, which is the best known of the alkaloids obtained from the Colchicum plant, is used as a drug of choice in the treatment of Gout Disease, Familial Mediterranean Fever, Behçet's Disease because it has anti-inflammatory properties and is known to have antitumor properties (Akbulut, 2009; Brossi, 1990). Anticholinesterase, isoquinoline alkaloids and phenolic acids such as coumaric acid, ferulic acid, kaffeic acid, vanillic acid, 2-hydroxybenzoic acid have been isolated from different *Colchicum* species (Azadbakht et. al., 2020).

In this study, the antimicrobial activities of ethanolic extracts prepared using bulb, leaves and flowers of *Cyclamen coum* species and corm and aerial parts of *Colchicum turcicum* and *Colchicum bornmuelleri* were investigated against test microorganisms by disk diffusion method. In addition, it was aimed to determine the antioxidant activities and total phenolic contents of the extracts obtained by DPPH scavenging method.

2. Material and Methods

2.1. Material: *Cyclamen coum* Mill. and *Colchicum turcicum* Janka species used in this study were collected from the under forest vegetation of Çilekli village (Sakarya). *Colchicum bornmuelleri* Freyn species was collected from the Tuzla Village (Sakarya)(see Fig. 1). The plants were washed and left to dry in the shade at room temperature for seven days.

Figure 1. A: *Cyclamen coum*, B: *Colchicum turcicum*, C: *Colchicum bornmuelleri*

All the chemicals and reagents (Ethanol, Methanol, Gallic Acid, Ascorbic Acid, Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Triptic Soy Broth, Mueller Hinton Agar (MH Agar) and Sodium Carbonate) used in the study were of the analytical grades and obtained from SIGMA company Germany.

2.2. Preparation of extracts: All the milled plant parts were placed in screw-capped glass bottles to be 100 g and 100 ml of

ethanol was added on them. These blends were mixed in a magnetic stirrer for 72 hours. A rotary evaporator was used at 40-45 °C to remove plant extracts from the solutions. For antimicrobial activity experiments 6400 µg/disk and for antioxidant activity and total phenolic content 1000 µg/mL ethanol extract stocks were prepared.

2.3. Disc diffusion method: All the strains of bacteria used in this study were purchased from Microbiology Research Laboratory of Sakarya University. To determine the antimicrobial activities of the extracts Disk Diffusion Method was used. 15 µL of the plant extracts prepared were taken and absorbed into sterile empty discs. The discs were allowed to dry for 2 hours at room temperature in the dark. 0,5 Mcfarland density suspension was prepared with previously activated microorganisms by using a densitometer. Inoculation was carried out in a sterile environment with a swab from prepared microorganism suspensions to MHA media. The discs impregnated with plant extract were left on the planted MHA medium with forceps and incubated at 37 °C for 24 hours. Ethanol impregnated discs were used as negative control, and Gentamicin loaded discs were used as positive control. After 24 hours, the antibacterial effect of plant extracts against microorganisms was evaluated by measuring the inhibition zone diameters around the disc using a digital caliper.

2.4. Antioxidant activity: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated according to the Blois (1958) method with minor modifications. Briefly, 1 mL of ethanolic extracts prepared in different concentrations was taken and 1 mL of 0.04 % solution of DPPH radical in ethanol was added on it. After mixing with vortex, it was kept at room temperature in the dark for 30 minutes and absorbances were recorded at 517 nm in the spectrophotometer. For the control, 1 mL of DPHH was added to 1 mL of ethanol solution. The percentage of DPPH scavenging activity of the extract was calculated using the following equation:

%DPPH radical scavenging = [(control absorbance- extract absorbance)/control absorbance] x 100

2.5. Total phenolic content: Total amount of phenolic content was determined by making minor changes in the Folin-Ciocalteu method of Singleton and Rossi (1965). 100 µL of the prepared extract was taken, 200 µL of 50% Folin-Ciocalteu reagent was added and left for 2 minutes. 1 mL of 2% sodium carbonate solution was added and the absorbance was read at 76 nm after one hour of incubation at room temperature in the dark. The total phenolic content was determined in mg / 100 g using Gallic Acid Standard.

2. Results and Discussion

The emergence of multidrug resistance in human and animal to pathogenic bacteria, as well as the undesirable side effects of some antibiotics, has increased interest in researching new antimicrobial drugs of plant origin. Traditionally used herbs produce a variety of compounds with known therapeutic properties. Plant-derived antimicrobial compounds can be formed in the branches, roots, leaves, bark, flowers or fruits of the plants (Ahmad and Beg, 2001; Albayrak and Kaya, 2019; De zoysa et. al., 2019). Considering the great potential of plants that are sources of antimicrobial drugs, this study will make significant contributions to the literature. In our study, the antimicrobial activities of extracts obtained from Cyclamen coum bulb, leaf and flower parts, Colchicum bornmuelleri and Colchicum turcicum from corm and aerials parts were evaluated, and the results are given in Table 1.

Table 1. Antimicrobial activity of extracts used in the study on test microorganisms

Samples (6400 µg/disk)		Test microorganism (Inhibision zone diameters, mm±SD)						
		Bs	**Ec**	**Ef**	**Se**	**Sa**	**St**	**Ca**
C. turcicum	Aerial parts	0	0	16.5±0.3	0	0	0	-
	Corm	0	7±0.3	0	0	0	0	-
C. bornmüelleri	Aerial parts	0	0	10.5±0.8	0	0	0	-
	Corm	0	7±0.7	0	0	0	0	-
C. coum	Bulb	0	0	8	0	0	0	8±1.0
	Leaf	0	0	0	0	0	0	0
	Flower	0	0	0	0	0	0	11±0.3
P. control	Gc	17	19	20	21	20	21	-
	Amp	-	-	-	-	-	-	16
N. control	Etanol	0	0	0	0	0	0	0

Ec*: Escherichia coli*, Se: *Staphylococcus epidermidis*, Ef: *Enterococcus faecalis*, Ca: *Candida albicans*, Bs: *Bacillus subtilis*, Sa: *Staphylococcus aureus*, St: *Salmonella typhimurium*, Gc: Gentamicin, Amp: Amphotericin B, - no tested.

The results revealed that aerial part extracts of both *Colchicum* species had moderate antibacterial effect against *E. faecalis* bacteria. Aerial extracts of *C. turcicum* and *C. bornmuelleri* species did not show antibacterial activity against other test microorganisms. The aerial extracts of the same plant species had showed a low level of antimicrobial effect against E. coli bacteria only.

The study revealed that the ethanolic extract prepared using all parts of *Colchicum balaense* plant created an inhibition zone diameter of 10 mm on *S. aureus*, 8 mm on *E. faecalis* and 9 mm on *E. coli*. (Mammadov et. al., 2009). In our study, it was observed that extracts obtained from the corm parts of *Colchicum* species formed 7 mm on *E. coli* and the extracts obtained from the aerial parts formed 10.5 mm and 16.5 mm inhibition zone diameters on *E. faecalis*. The difference between the studies may be due to the different plant species, as well as the place where the plants are collected, extract preparation methods, and the use of different plant parts while preparing the extract. As similar as our study, Hanif et al. (2010) reported that extracts obtained from *Colchicum autumnale* L. species did not show antimicrobial activity on *B. subtilis* bacteria. Türker and Usta (2008) reported that aqueous extracts of *Cyclamen coum* leaves did not show antibacterial effects on *E. coli, P. aeruginosa*, *S. epidermidis* and *S. aureus*. In our study, it was determined that *Cyclamen coum* leaf extracts did not show antimicrobial activity on the test microorganisms used.

Semerci et al. (2019) reported that methanolic extracts of *Cyclamen coum* flowers created an inhibition zone diameter of 9.5 mm on *C. albicans*. Similarly, it was observed that ethanol flower extracts created an inhibition zone diameter of 11 mm on *C. albicans*. Low antimicrobial activity does not mean that the plant does not contain bioactive compounds or that the plant has no activity against microorganisms. The fact that the extracts contain insufficient amounts of active ingredient to show antimicrobial activity can also cause negative results. Medicinal plants are generally rich in flavonoids, tannins, coumarins, lignans and phenolic compounds. The antioxidant properties of polyphenols are due to their redox properties such as being a reducing agent and hydrogen donor, acting as a metal chelator, and binding reactive oxygen. Polyphenolics exhibit a wide variety of biological effects such as antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic (Piluzza and Bullitta, 2011; Ben Yakoub et. al., 2018). In this study, it was determined that aerial part extracts of *Colchicum* species have higher antioxidant activity and total phenolic

content (Table 2). The highest antioxidant activity was found in the extract prepared from the aerial part of the *Colchicum turcicum* plant. The lowest antioxidant activity was observed in the extracts obtained from the corm parts of *Colchicum bornmuelleri* plant.

Table 2. Antioxidant activities and total phenolic contents of the extracts

Samples	TPC(mgGA/100g)±SD	IC_{50} µg/mL(±SD)
C. bornmuelleri aerial parts	191.85±1.2	23.78±2.6
C. bornmuelleri corm	17.76±0.6	768.65±1.0
C. turcicum aerial parts	162.2±2.5	14.2±3.0
C. turcicum corm	13.3±1.3	530.92±5.9
C. coum bulb	43.41±0.2	145.15±1.1
C. *coum* leaf	46.4±0.8	151.68±1.9
Ascorbic acid	-	3.6±0.1

Kılıç et al. (2014) calculated the IC_{50} values of water and acetone extracts obtained from the root and stem parts of the *Colchicum turcicum* plant as 29.98 µg/mL and 45.74 µg/mL, respectively. In the same study, the total phenolic content of the extract prepared with water was 0.454 mg/g, and the total phenolic content of the extract prepared with acetone was 2.172 mg/g. In our study, the IC_{50} value of the aerial extract prepared with ethanol was calculated as 530.92 µg/mL and the total phenolic value was calculated as 13.3 mg GA/100 g. The differences between these studies may vary according to the place where the plant is collected, the solutions used in the extract preparation and the plant parts used. Mammadov et al. (2009) examined the antioxidant activities of extracts obtained from the corm and aerial parts of the *Colchicum balaense* plant by using percentage of DPPH scavenging ratio. At the end of the study, it was reported that the extracts obtained from the aerial parts of the *C. balaense* plant showed higher antioxidant activity. In another study, the scavenging rate of the ethanolic corm extracts prepared from *Colchicum autumnale* plant was 34% and the scavenging rate of flower extracts was 52% (Suica-Bungez et al., 2017). Similar to the literature data our study revealed that the aerial parts of *Colchicum* species show higher antioxidant activity compared to the corm parts.

Jaradat et al. (2017) calculated the IC50 value as 31 µg/mL and the total phenolic content amount as 32.7 mg GA/100 g in the methanol extracts they obtained using the aerial parts of the *Cyclamen coum* plant. In this study, IC_{50} value of *Cyclamen coum* plant leaf extracts prepared with ethanol was calculated as 151.68 µg/mL and total phenolic content amount as 46.4 mg GA/100 g. While the total amount of phenolic matter was similar in those two studies, it was observed that there were differences in DPPH scavenging activity which could be linked with differences in the used plant parts and type of the extracting agent.

4. Conclusions

The study revealed that the aerial parts of *Colchicum turcicum* and *Colchicum bornmuelleri* species showed higher antioxidant activity compared to the ascorbic acid as the standard antioxidant. It

is thought that these herbs can be used in a wide variety of fields such as the pharmaceutical industry, food supplements and food preservatives as potential sources of antioxidants. Although it is seen that the plants used in the study do not have high antimicrobial activity, we think that it is important to clarify the chemical structures by purifying the contents of bioactive substances with different solutions.

Acknowledgements
Thanks Dr. Mehmet Sağıroğlu for identification of plants

Conflict of Interest
The authors declare that they have no conflict of interest.

References

1. Ahmad, I. Beg, A.Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 74: 113–123.
2. Akbulut, S. 2009. Hamsiköy Yöresinde Odun Dışı Bitkisel Ürün Olarak Alchemilla Spp. Ve Colchicum Speciosum' un Envanteri Üzerine Bir Araştırma. Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Orman Mühendisliği Bölümü, Doktora Tezi.
3. Albayrak, S. Kaya, O. 2019. Antioxidant, Antimicrobial and Cytotoxic Activities of Endemic Astragalus argaeus Boiss. from Turkey. Hacettepe J. Biol. & Chem., 47 (1): 87–97
4. Azadbakht , M. Khoshvishkaie, E., Davoodi, A., Hosseinimehr, S.J., Azadbakht, M., Emami, S., Jouybari, H.B., Mirzae, F., Ghadiri, K. 2020. Optimization of Ultrasound-Assisted Acidic-Solvent Extraction of Colchicine from Colchicum kurdicum (Bornm.) Stef. Using Response Surface Methodology, Research Journal of Pharmacognosy ,7(3): 5-13
5. Ben Yakoub,A.R. Abdehedic, O., Jridic, M.,Elfallehd, W., Nasric, M., Ferchichi,A. 2018. Flavonoids, phenols, antioxidant, and antimicrobial activities in variousextracts from Tossa jute leave (Corchorus olitorus L.). Industrial Crops & Products 118: 206–213
6. Blois MS, (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181 (1958) 1199. 13
7. Bokov, D. O. Krasikova, M. K., Sergunova, E. V., Bobkova, N. V., TYu, K., Bondar, A, Marakhova A, Morokhina S, Krasnyuk I, Moiseev D. 2020. Pharmacognostic, Phytochemical and Ethnopharmacological Potential of Cyclamen coum Mill. Pharmacognosy Journal, 12(1): 204-212.
8. Brossi, A. 1990. Bioactive Alkaloids. 4. Results of Recent Investigations with Colchicine andPhysostigmine., Journal Of Medicinal Chemistry, 33(9).
9. Çaliş, İ. Yürüker, A., Tanke,r N., Wright, A.D., Sticher, O. 1997. Triterpene Saponins from Cyclamen coum var. coum. Planta Med., 63(2): 166-70.
10. Çoban, Ö.E. Batır, B. 2010. Antioksidan Etkili Bazı Bitki ve Baharatların Gıdalarda Kullanımı, Electronic Journal of Food Technologies 5(2) 7-19.
11. Davis, P.H. 1978 Flora of Turkey and The East Aegean Islands, 6. Cilt, Edinburgh Univ. Press, London, 128–135.
12. De Zoysa, M.H.N. Rathnayake, H., Hewawasam, R.P., Wijayaratne, W.M.D.G.B. 2019. Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. Hindawi International Journal of Microbiology
13. Düşen, O.D., 2005. Akdeniz Bölgesi'nde Yayılış Gösteren Colchicum L. (Liliaceae) Cinsine Ait Türlerin Taksonomik Yönden Araştırılması. Akdeniz Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi.
14. Güner, A., Aslan, S., Ekim, T., Vural, M. ve Babaç, M.T. (edlr.). 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul
15. Hanif, M.A. Bhatti, H.N., Jamil, M.S., Anjum, R.S., Jamil, A., Khan, M.M. 2010. Antibacterial and Antifungal Activities of Essential Oils Extracted from Medicinal Plants Using CO2 Supercritical Fluid Extraction Technology Asian Journal of Chemistry, 22(10): 7787-7798.
16. İnceçayır, D. Semerci, A.B., Mustafa, N., Tunç, K. 2019. Catalpa bignonioides Metanolik Çiçek Ekstraktının Biyolojik ve Kimyasal Aktivitesi. Türk Tarım ve Doğa Bilimleri Dergisi, 6(2): 230–234.
17. Jaradat, N.A. Al-Masri, M., Hussen, F., Zaid, A.N., Ali, I., Tammam, A., Odeh, D.M., Shakarneh, O.H., Rajabi, A. 2017. Preliminary Phytochemical and Biological Screening of Cyclamen coum a Member of Palestinian Flora. Pharmaceutical Sciences, 23, 231-237.
18. Kılıç,I. Yesiloglu, Y., Bayrak, Y. 2014. Investigation on the Antioxidant Activity of Roots and Stem of Colchicum turcicum L. Asian Journal of Chemistry; 26(1), 5-9.

19. Mammadov, R. Düşen, O., Demir, D.U, Köse, E. 2009. Antioxidant and antimicrobial activities of extracts from tubers and leaves of Colchicum balansae Planchon. Journal of Medicinal Plants Research ,3(10): 767-770.
20. Piluzza, G. Bullitta, S. 2011. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharmaceutical Biology, 49(3): 240–247.
21. Semerci, A.B. Inceçayir, D., Tunç, K., Sağıroğlu, M. 2019. Antimicrobial Activity of Different Flower Extracts. Curr. Pers. MAPs, 2: 53-58.
22. Singleton VL, Rossi JA, (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am J Enol Viticult, 16 (2017) 144.
23. Suica-Bunghez, I.R. Ion, R.M., Teodorescu, S., Sorescu, A.A., Stirbescu, R.M., Stirbescu, N.M. 2017. Fitochemical And Antioxidant Characterization Of autumn Crocus (Colchicum Autumnale) Flowers and Roots Plant Extracts. Journal of Science and Arts, 3 (40), 539-546.
24. Tanrıverdi O, D. 2019. Yalova İli Geofitleri ve Peyzajda Kullanım Olanakları. Bursa Uludağ Üniversitesi, Fen Bilimleri Enstitüsü, Peyzaj Mimarlığı Bölümü, Yüksek Lisans Tezi.
25. Türker, A. U. Usta, C. 2008. Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. Natural Product Research, 22 (2):136–146.
26. Yaylı, N. Baltaci, C., Zengin, A., Küçükislamoglu, M., Genç, H. 1998. Triterpenoid saponin from Cyclamen coum. Phytochemistry, 48(5): 88, 1-4