International Journal of Secondary Metabolite
2022, Vol. 9, No. 2, 149–157
https://doi.org/10.21448/ijsm.1056920
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Antiproliferative and antioxidant potential of methanol extracts of aerial parts of *Colchicum boissieri* and *Colchicum balansae*

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Abstract: The antiproliferative and antioxidant activities, and total phenolic, flavonoid, and tannin contents of methanol extracts obtained from the aerial parts of *Colchicum boissieri* and *Colchicum balansae* were investigated in the present study. The evaluation of the antiproliferative effects of the extracts under study was carried out using the MTT assay. The antioxidant potentials of the extracts were assigned by using several assays (FRAP, CUPRAC, DPPH, ABTS, and *β*-carotene). The antiproliferative effects of both extracts on Caco-2 cells appeared to be generally similar for the incubation times tested. The antioxidant potential was found to be higher in the extract of *C. boissieri* (FRAP: on average 1.39 mg TE/g, CUPRAC: on average 10.06 mg TE/g, DPPH: on average IC_{50}= 0.59 mg/mL, ABTS: on average IC_{50}= 0.267 mg/mL, *β*-carotene: on average 78.58%) than the extract of *C. balansae*. In terms of total phenolic, flavonoid and tannins contents, *C. boissieri* extract (on average 1.97 mg GAE/g, 8.65 mg QE/g and 4.75 mg CE/g, respectively) was determined to be richer than *C. balansae* extract. The results suggest that both extracts have some biological properties for pharmaceutical applications. Further studies may contribute to the use of these plants for various purposes such as natural antioxidant sources or cancer agents.

ARTICLE HISTORY
Received: Jan. 12, 2022
Revised: Feb. 18, 2022
Accepted: Apr. 26, 2022

KEYWORDS
Colchicum boissieri,
Colchicum balansae,
Methanolic extract,
Antiproliferative,
Antioxidant

1. INTRODUCTION

Cancer is a multifactorial disease that has specific features at different levels as cellular, tissular, and organismic (Paul, 2020). Cancer is one of the leading causes of death all over the world. According to Sung *et al,* (2021), female breast cancer is the most diagnosed cancer followed by lung, colorectal, prostate, and stomach cancer. In addition, lung cancer continues to be the leading cause of cancer death followed by colorectal, liver, stomach, and female breast cancers. Many anticancer drugs were reported to have a narrow therapeutic potential because of their systemic toxicity and lack of selective properties against tumor (Kratz *et al.,* 2008).

Free radicals are generated by various metabolic processes and the uncontrolled or increased formation of free radicals in the body may lead to oxidative stress (Alkadi, 2020). The balance between free radicals and antioxidants are important for health (Lobo *et al.*2010). Oxidative

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ISSN-e: 2148-6905 / **© IJSM 2022**

stress has been known to contribute to various diseases such as cancer, atherosclerosis, diabetes, neurological disorders, and hypertension (Birben *et al.,* 2012). Antioxidants have the ability to neutralize free radicals and supply protection against damage owing to these free radicals (Zehiroglu & Sarikaya, 2019). Not only do plants have the potential for preventing or treating diseases but also they are of great interest as a good source for discovering new pharmaceutical drugs (Parasuraman, 2018). Plants contain secondary metabolites with various biological activities such as anti-inflammatory and anticancer, and have an important place in obtaining products with fewer side effects and higher efficiency in cancer treatment. In this context, screening of crude extracts of plants is important to provide natural agents with anticancer properties (Kooti *et al.,* 2017). In addition, plants or plant-based products, such as vegetables, flowers, fruits, cereals, and spices, have been known as the source of natural antioxidants. Considering their effects on human health, the evaluation of the antioxidant potentials of plants for different applications, including food additives, remains in interest (Xu *et al.,* 2017; Lourenço *et al.,* 2019).

Turkey is rich in terms of the *Colchicum* L. species it possesses. *Colchicum* species that are one of the significant plants with medicinal properties belong to Colchicaceae family and are evaluated in two groups in terms of flowering time in autumn or in spring. *Colchicum* genus contains species which are rich in alkaloids, and colchicine is the main alkaloid of this genus. Colchicine has been reported to have anticancer and anti-inflammatory effects, to be important in the treatment of several diseases such as gout, Familial Mediterranean Fever, psoriasis and other dermatological disorder, and also have a limited therapeutic index (Al-Snafi, 2016; Toplan *et al.,* 2016).

There have been studies on antioxidant activities of the extracts of the *C. boissieri* Orph. and *C. balansae* Planch. (Mammadov *et al.,* 2009; Sevim *et al.,* 2010), which are autumn-flowering species from Turkey (Toplan *et al.,* 2016). However, no literature is available about the effects of methanol extracts of aerial parts of plants in question on the cell viability of Caco-2 (human colorectal adenocarcinoma) cells. The present study, therefore, aims to contribute to the literature about antiproliferative effects against colorectal human cancer cell line, and also antioxidant properties as well as the amount of total phenolic, flavonoid and tannin contents of their methanolic aerial part extracts.

2. MATERIAL and METHODS

2.1. Chemicals and Reagents

The chemicals and reagents were provided from Biochrom (GmbH, Germany), Capricorn Scientific (GmbH, Germany), and Applichem (Germany) for cell culture assay and from Sigma-Aldrich (Germany) for other experiments.

2.2. Plant Materials and Preparation of the Plant Extracts

The aerial parts of *Colchicum boissieri* (1400 m, Yılanlı Mountain) and *Colchicum balansae* (30 m, Ula) were obtained from the Muğla province, Turkey in October 2021. An expert taxonomist Dr. Olcay Ceylan at Muğla Sıtkı Koçman University, Muğla, Turkey, carried out authentication of the plant specimens. Herbarium specimens of these plants were kept in the herbarium of Biology Department at Muğla Sıtkı Koçman University. After the plant parts dried in shadow at room temperature were milled using a blender, about 10 g of plant sample was soaked in 100 mL of methanol, and then shaked at 55°C for 24 h. Each extract solution was filtered, followed by the removal of methanol using a rotary evaporator (IKA RV10D, Staufen, Germany). After lyophilization, the extracts obtained were deposited at -20°C in the dark (Turan & Mammadov, 2018).

2.3. Cell Culture

Colorectal (Caco-2, ATCC) human cancer cell line was grown in Roswell Park Memorial Institute (RPMI)-1640 medium which was supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were cultured at 37°C in a humidified incubator with 5% CO_2 and monitored daily using an inverted microscope (Zeiss, Germany).

2.4. MTT Assay

The antiproliferative activities of the plant extracts were assigned based on MTT assay (Mosmann, 1983) with some modifications for 24 h and 48 h. Plant extracts were separately dissolved in 10% DMSO (Dimethyl sulfoxide) to obtain stock solution and diluted with cell growth medium before addition to the cells. The seven serial dilutions of the extracts (0.02-1.2 mg/mL) were used in this assay. To detect the cell viability, the cells at $2x10^4$ cells/well were seeded into 96 well plates. After incubation at 37°C for 24 h, the cells were exposed to different concentrations of plant extracts for 24 h and 48 h. Cells not exposed to any extract were control cells. After each incubation time, the medium in each well was replaced by 100 µL of fresh growth medium and 10 µL of MTT (5 mg/mL) was added to each well, followed by 4 h incubation. 100 µL of DMSO were added to each well after removing the medium. The microplates were shaken at 150 rpm for 6 min and the absorbance was recorded at 540 nm using a microplate reader (ThermoScientific, Multiscan FC, USA). Cell viability was expressed as a percentage cell viability by considering the absorbance of the treated and control cells.

2.5. Antioxidant Potential of the Plant Extracts

Ferric ion reducing antioxidant power (FRAP) assay and Cupric ion reducing antioxidant capacity (CUPRAC) assay were done based on the previous methods of Benzie and Strain (1996) and Apak *et al*. (2006), respectively and the outcomes were expressed as milligram of Trolox equivalent per gram of the extract (mgTE/g). For the investigation of DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity of the extracts, the methods stated by Turan and Mammadov (2018) and Re *et al.* (1999) were followed, respectively, and the results are presented as IC_{50} values. *β*-carotene–linoleic acid method used for evaluation of the antioxidant capacity of the extracts was conducted taking into account the method described by Amarowicz *et al.* (2004), and also the total antioxidant activity was calculated according to the formula of Amarowicz *et al. (*2004) and expressed as a percentage.

2.6. Total Phenolic, Flavonoid and Tannin Contents

The total phenolic, total flavonoid, and total tannin contents of the extracts were evaluated by referring to the methods described by Turan and Mammadov (2018), Aryal *et al*. (2019), and Bekir *et al.* (2013). The results are presented as equivalents of Gallic acid (mgGAE/g), Quercetin (mgQE/g), and Catechin (mgCE/g) in milligrams per g of the extract, respectively.

2.7. Data Analysis

The SPSS software (version 22.0 for Windows, IBM Corp., Armonk, N.Y., USA) was used to evaluate the results and compute the IC_{50} (half maximal inhibitory concentration) values. The results obtained from at least three separate experiments were expressed as mean ± SE (Standard Error). The statistical analyses of data were done with ANOVA (Analysis of Variance) and then Tukey multiple comparison test ($p<0.05$). A t-test was used when there were only two groups. The graphs were presented using the GraphPad Prism 7.0 Software program.

3. RESULTS and DISCUSSION

3.1. Antiproliferative Activity of Plant Extracts

MTT assay, which is basically based on the conversion of MTT into insoluble purple-colored formazan crystals by viable cells, is one of the commonly used colorimetric assays. In addition, DMSO is one of the solvents used to dissolve the formazan crystals before recording the absorbance (Kamiloglu *et al.,* 2020). The effects of the extracts on the viability of Caco-2 cells were determined using MTT assay and the results are shown in Figure 1 and IC_{50} values are also presented in Table 1. Given these results, the antiproliferative effects of both extracts on Caco-2 cells appear similar.

Figure 1. Antiproliferative activity of the *Colchicum boissieri* (a) and *Colchicum balansae* (b) extracts on Caco-2 cell line. Statistical analysis for results of MTT assay at each incubation time was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

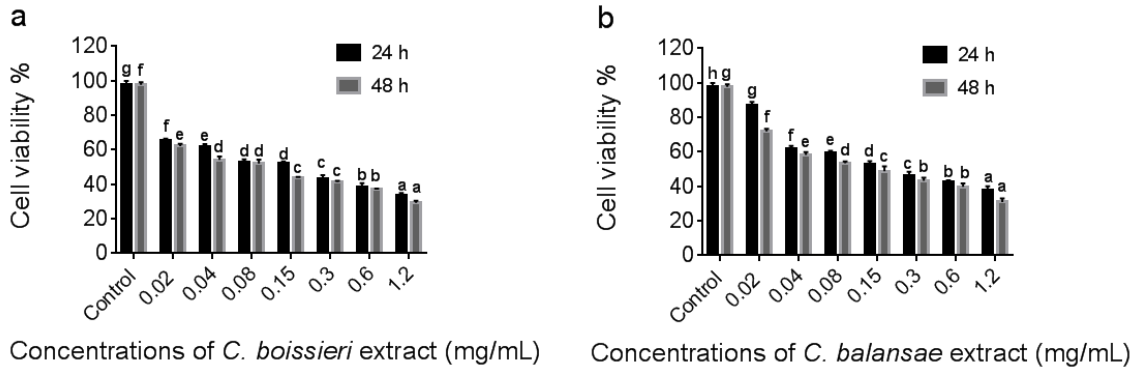

Table 1. Approximate IC_{50} values of the *Colchicum boissieri* and *Colchicum balansae* extracts

	IC_{50} values (mg/mL)	
Plant extracts	24 h	48 h
C. boissieri	0.2	0.1
C. balansae	0.217	0.131

Pırıldar *et al.* (2010) reported that all extracts, except for the seed extract, exhibited more cytotoxic activity against the promyeloid leukemia cell line (HL60) than against the chronic myeloid leukemia cell line (K562), among methanol extracts obtained from the different parts of *C. baytopiorum* CD Brickell. The highest cytotoxic activity observed on HeLa (cervical cancer) cell line was declared to originate from the methanolic extract of cormus of *C. sanguicolle* K.M. Perss among different plant extracts tested (Artun *et al.,* 2016). Becer *et al.* (2019) stated that the extract of *C. pusillum* Sieber at all concentrations tested for 24 h and 48 h caused the toxic effect against Colo-320 (human colon adenocarcinoma) cell in a dose and time-dependent manner. Also, other previous studies reported the antiproliferative activity of the extracts obtained from different *Colchicum* species (Ozsoylemez *et al.,* 2016; Gulsoy-Toplan *et al.,* 2018; Dagdeviren Ozsoylemez & Ozcan, 2021). However, according to available literature, the present study is most likely the first study based on the antiproliferative effects of the *C. boissieri* and *C. balansae* extract on Caco-2 cell line.

3.2. Antioxidant capacity of plant extracts

Antioxidant activities of the methanol extract of aerial parts of *C. boissieri* and *C. balansae* were evaluated by different assays and the results are shown in Table 2. The antioxidant activity of the extract of *C. boissieri* was determined to be better than the extract of *C. balansae*. Considering the reducing potential, FRAP, and CUPRAC activities for *C. boissieri* extract were 1.39±0.03 and 10.06±0.73 mg TE/g, respectively. In addition, according to the results of DPPH,

ABTS and β-carotene/linoleic acid assay, the antioxidant activities of both extracts were low compared to that of BHA (Butylated hydroxyanisole) used as standard. The IC_{50} values of DPPH and ABTS radical scavenging tests for *C. boissieri* extract were 0.59±0.018 and 0.267±0.008 mg/mL, respectively. Also, the highest antioxidant activity between the plant extracts was detected as 78.58±4.64% for *C. boissieri* extract using the β-carotene/linoleic acid method.

Table 2. Antioxidant activity of *Colchicum boissieri* and *Colchicum balansae* extracts.

Plant extracts	FRAP (mg TE/g)	CUPRAC (mg TE/g)	DPPH (IC_{50}, mg/mL)	ABTS (IC_{50}, mg/mL)	β-carotene/ linoleic acid (%)
C. boissieri	1.39±0.03a	10.06±0.73a	0.59±0.018b	0.267±0.008b	78.58±4.64b
C. balansae	0.65±0.03b	3.59±0.58b	1.43±0.049c	0.487±0.095c	69.58±3.81c
BHA	nt	nt	0.019±0.003a	0.008±0.004a	88.41 ±0.36a

The values are presented as mean ± standard error (SE). The different lowercase letters in the same column indicate significant differences. nt: not tested

Antioxidants are capable of decreasing oxidative stress, therefore they have a critical function in stopping and curing ailments, sustaining health. Many methods have been used for the analysis of antioxidant activity (Munteanu and Apetrei, 2021). In the present study, the different assays; namely, FRAP, CUPRAC, DPPH, ABTS, and the β-carotene/linoleic acid method were conducted for the assessment of the antioxidant potential of the extracts.

DPPH and ABTS scavenging assays are commonly used simple spectrophotometric methods and the antioxidant activity is determined colorimetrically based on absorbance changes of stable colored radicals. The FRAP and CUPRAC assays, which are spectrophotometric methods, determine the capacity of an antioxidant to reduce an oxidant that changes colorimetrically when reduction occurs. The principles of FRAP and CUPRAC assays are the reduction of Fe^{3+}-ligand complex to Fe^{2+} and reduction of Cu^{2+} to Cu^{+}, respectively, by means of antioxidants (Pisoschi & Negulescu, 2011; Munteanu & Apetrei, 2021). Another common assay for the evaluation of the antioxidant activity of the plant extracts is the β-carotene bleaching assay. Briefly, the discoloration of β-carotene is observed due to radical species originating from the oxidation of linoleic acid and the presence of antioxidants can be delayed discoloration and the bleaching the color is spectrophotometrically measured with ease (Pisoschi & Negulescu, 2011; Xiao *et al.,* 2020). As a result of the assays aforementioned, the *C. boissieri* extract caused a higher antioxidant activity than that of *C. balansae* extract. In a previous study, the antioxidant activities of the extracts from tuber and leaf of *C. balansae* from Muğla province by using different solvents were examined using β-carotene bleaching method and DPPH scavenging activity assay. In the same study, the leaves extracts of *C. balansae* displayed higher antioxidant properties than those of tuber extract, and also the highest antioxidant activity efficiency and the highest free radical scavenging activity were detected in leaf ethanol (%64) and leaf benzene (68.35%) extract, respectively (Mammadov *et al.,* 2009). Unlike the current study, Sevim *et al.* (2010) declared that the DPPH scavenging activities of the methanol extracts from *Colchicum* taxon including *C. boissieri* and *C. balansae* tested by them were to be low below 40% at 2000 µg/mL. The antioxidant capacities of flower, root, and leaf extracts obtained from *C. szovitsii* subsp. *szovitsii* (Rocchetti *et al*., 2019) and *C. triphyllum* (Senizza *et al.,* 2020) obtained using different extraction procedures were higher than those in the present study in terms of FRAP and CUPRAC assays. The antioxidant activities of the extract of different *Colchicum species* for example *C. speciosum* Steven (Souri *et al.,* 2008), *C. turcicum* Janka (Kiliç *et al.,* 2014), *C. autumnale* L. (Suica-Bunghez *et al.,* 2017; Hailu *et al.,* 2021) were also examined.

3.3. Total Bioactive Compounds of Plant Extracts

The methanolic aerial part extract of *C. boissieri* was found to be richer than *C. balansae* extract in terms of total phenolic, flavonoid, and tannins contents (Table 3), with 1.97±0,07 mg GAE/g, 8.65±1.67 mg QE/g and 4.75±0.51 mg CE/g, respectively.

Table 3. Total phenolic, total flavonoid, and total tannin contents of *C. boissieri* and *C. balansae* extracts

Plant extracts	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total tannin content (mg CE/g)
C. boissieri	1.97±0.07a	8.65±1.67a	4.75±0.51a
C. balansae	1.05±0.05b	5.37±0.11a	3.00±0.24b

The values are presented as mean ± standart error (SE). The different lowercase letters in the same column indicate significant differences.

Natural antioxidants of plant origin such as phenolic acids, flavonoids, lignans, and tannins have been reported to show various biological activities such as antibacterial, antiaging, anticancer, anti-inflammatory, and antioxidant (Xu *et al.,* 2017). The higher antioxidant potential of *C. boissieri* extract may be attributed to the presence of bioactive compounds tested and found to be higher than that of *C. balansae* extract. The total phenolic contents of *C. boissieri* and *C. balansae* extract tested here were found to be lower than the previously reported value by Rocchetti *et al.* (2019) who determined that for the flower extracts, the highest total phenolic content was found to be in the aqueous macerated extract of flower of *C. szovitsii* Fisch. & C.A.Mey. subsp. *szovitsii* (40.70 mgGAE/g). The total flavonoid contents of water and acetone extract of *C. turcicum* (Kiliç *et al*., 2014) were recorded as 13.1 mg GAE/g and 83.9 mg GAE/g, respectively, and also the total phenolic contents of their extracts in question were expressed as catechol equivalents and varied from 0.454 mg CE/g (water) to 2.172 mg CE/g (acetone). Many researchers have also revealed the amount of the total bioactive contents of various *Colchicum* species extract; for example, C. *kurdicum* (Bornm.) Stef. (Azadbakht *et al.,* 2020), *C. autumnale (*Suica-Bunghez *et al*., 2017; Hailu *et al*., 2021), *C. speciosum*, C. *robustum* Stef. (Davoodi *et al*., 2021).

4. CONCLUSION

The results of the present study revealed that the extract of *C. boissieri* has more potential than that of *C. balansae* in terms of antioxidant activity. In addition, total bioactive contents evaluated are higher in the extract of *C. boissieri* than in the extract of *C. balansae*. The antiproliferative effects of both extracts on Caco-2 cells were generally observed to appear similarly. The current study is most likely the first study based on the total bioactive contents of extracts, especially *C. boissieri* and the antiproliferative effects of them on the Caco-2 cell line. Of the extracts assessed, *C. boisserie* is a relatively promising nominee for future investigations compared to *C. balansae*. Various biological studies to be carried out in future related to these species, including isolation and identification of their phytochemicals, may contribute to their application such as food additives, sources of natural antioxidants, and anticancer agents.

Acknowledgments

The author would like to thank Dr. Olcay Ceylan from Muğla Sıtkı Koçman University for providing plant samples and master's students Mehmet Özgür Atay and Burcu Şahin for their help with laboratory work.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM

belongs to the author(s). Ethics Committee Approval and its number should be given by stating the institution name which gave the ethical approval.

Authorship contribution statement

Mehlika Alper: Investigation, Analysis, and Writing - original draft

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