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Phenolic profile, antioxidant, and antiproliferative activities of *Convolvulus aucheri* Choisy

Fenolni profil, antioksidantno i antiproliferativno delovanje *Convolvulus aucheri* Choisy

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Abstract

Background/Aim. It is known that some members of the genus *Convolvulus (C)* L. are commonly used in Turkish folk medicine. These species are powerful in curing toothache and joint pains. Due to the limited information on the biological activities of *C. aucheri,* a species almost exclusively occurring in Turkey, we aimed to investigate the antioxidant and cytotoxic effects of three extracts obtained from the plant, as well as to characterize their phenolic profile. **Methods.** The antioxidant activity of the extracts was determined by using ABTS, NO, FRAP, phosphomolybdenum, and metal chelating assays. In addition, the bioactive compounds found in the extracts, such as total phenolics, flavonoids, and saponins, were determined. Cytotoxicity was assessed by using the CellTiter-Glo assay on HeLa and H1299 cancer cells. **Results.** The methanol extract of *C. aucheri* demonstrated the highest antioxidant activity as well as the highest phenolic, flavonoid, and saponin content. The high-performance liquid chromatography (HPLC) analysis showed that the major phenolic compounds in the extract were chlorogenic acid, (+)-catechin, rosmarinic acid, and rutin. The methanolic extract obtained from the aerial parts of *C. aucheri* was found to interfere with the viability of HeLa cells, with an IC_{50} value of 14.22 μg/mL being recorded. **Conclusion.** Our results showed that *C. aucheri* could be a good candidate as a novel and alternative natural antioxidant and antitumor source.

Key words:
antioxidants; convolvulus; chromatography, high-pressure liquid; phytotherapy; turkey.

Apstrakt

Uvod/Cilj. Poznato je da se neke biljne vrste roda *Convolvulus (C)* L. često koriste u turskoj narodnoj medicini kao snažni agensi u lečenju zubobolje i kod bolova u zglobovima. Zbog ograničenih informacija o biološkim aktivnostima *C. aucheri*, vrste koja se gotovo isključivo javlja u Turskoj, cilj rada bio je ispitivanje antioksidativnog potencijala, kao i citotoksičnih svojstava tri različita ekstrakta, uz karakterizaciju njihovog fenolnog profila. **Metode.** Antioksidativna aktivnost ekstrakta određena je korišćenjem ABTS, NO, FRAP, fosfomolibdenskog i testa heliranja metala. Pored toga, u ekstraktima su identifikavana i određena sledeća bioaktivna jedinjenja: ukupni fenolni, flavonoidi i saponini. Citotoksičnost je procenjena primenom *CellTiter-Glo* testa na kancerskim HeLa i H1299 ćelijskim linijama. **Rezultati.** Metanolni ekstrakt *C. aucheri* ispoljio je najveću antioksidativnu aktivnost, a u njemu je utvrđen i najveći sadržaj fenola, flavonoida i saponina. Tečnom hromatografijom pod visokim pritiskom (HPLC) utvrđeno je da su glavna jedinjenja u ovom ekstraktu hlorogenska kiselina, (+)-katehin, ruzmarinska kiselina i rutin. Otkriveno je da metanolni ekstrakt dobijen iz nadzemnih delova *C. aucheri* smanjuje vitalnost HeLa ćelija, a određena IC_{50} vrednost bila je 14,22 μg/mL. **Zaključak.** Naši rezultati su pokazali da bi *C. aucheri* mogao biti dobar novi i alternativni izvor prirodnih antioksidanasa i jedinjenja sa citotoksičnim efektom.

Ključne reči:
antioksidansi; convolvulus; hromatografija, tečna, pod visokim pritiskom; fitoterapija; turska.

Introduction

Natural products, especially plant derivatives, are a potential supply for novel biologically active compounds [1]. The herbal products have become very popular, especially because of the negative effects of synthetic drugs [2]. Natural resources with their rich polyphenolic contents and different types of secondary metabolites could be potential antioxidant

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and anticancer agents. Antioxidants are able to prevent or postpone the oxidative injury, which causes several diseases, by intervening with the formation of free radicals or defusing them whereafter they are produced. In most cases, natural compounds are more effective and more reliable than synthetic antioxidants. For this reason, there is a growing interest in the identification of natural components that are useful for humans [3].

The genus *Convolvulus (C)* L. belongs to the Convolvulaceae family, including 250 taxa, generally recognized as bindweeds. With respect to recent research, this genus is represented by 39 taxa (three of them hybrids) in Turkey [4]. Some taxa of this genus are used in the treatment of toothache and also as anthelminthic, laxative, and cholagogue in Turkish folk medicine [5]. *C. aucheri* Choisy is located in a narrow area in the southern part of Turkey**.** The observations made in field studies and the fact that *C. aucheri* could be collected from only one area contributed to evaluating the threat category of this species as Vulnerable (VU) according to the IUCN (International Union for Conservation of Nature) [6].

Extracts of various members of *Convolvulus* have been demonstrated to have antioxidant, anticancer, and antinociceptive activities [7–9]. Although some studies have been performed on the phytochemicals and biological activities of *C. arvensis*, *C. pluricaulis*, and *C. fatmensis* [7, 10, 11], only one study has been performed with *C. aucheri* [12]. Therefore, the aim of this research was to reveal the antioxidant potentials and total bioactive compounds (phenolic, flavonoid, and saponin) of *C. aucheri*, as well as their cytotoxic effect on HeLa and H1299 cancer cells. In this research, the phenolic components of *C. aucheri*, which were collected from Turkey, were also determined *via* reversed-phase high-performance liquid chromatography (RP-HPLC).

Methods

Plant material and extraction

The individuals of *C. aucheri* were collected in the flowering stage from Hatay-Turkey at NATO Radar Station in Kisecik, serpentine slopes, ca 885 m. Taxonomic identification of the plant was confirmed by the senior taxonomist Dr. Candan Aykurt in the Department of Biology, Akdeniz University, Antalya-Turkey. The voucher specimen was deposited at the Akdeniz University Herbarium (Voucher no: C. Aykurt 2665).

The plants were air-dried, and their aerial parts were powderized. Each of the powdered plants (10 g) was separately extracted with 100 mL of methanol, acetone, and petroleum benzine in a shaker water bath for 6 hrs at 55 °C. The extracts were filtered and vaporized using a rotary evaporator, and the methanol: water (70 : 30, v/v) extract was lyophilized due to the residual water content. The highest extraction yield was obtained with methanol (36.11%). The yields of the acetone and petroleum benzine extracts were 21.25% and 10.64%, respectively. The crude extracts were kept at +4 °C until needed.

Total phenolic content (Folin–Ciocalteu assay)

The total phenolic content of the extracts was analyzed via the Folin-Ciocalteu method, in which gallic acid was used as a standard [13]. One mL of extract solution was added to 46 mL of distilled water and 1 mL of Folin-Ciocalteu reagent and was mixed properly. After 3 min, the mixture was mixed with 3 mL of sodium carbonate (2%) and shaken intermittently for 2 hrs. The absorbance was measured at 760 nm, and the total phenolic constituent was determined as gallic acid equivalents (mg GAEs/g).

Total flavonoid content

The total flavonoid content of the extracts was determined by the aluminum colorimetric method [14]. One mL extract was mixed with 1 mL of 2% aluminum trichloride ($AlCl_3$) in methanol. After a 10 min incubation at 25 °C, the absorbance was read at 415 nm. The total flavonoid content was expressed as quercetin equivalent (mg QE/g).

Total saponin content

Total saponin content was determined by the vanillin-sulphuric acid method [15]. The extracts were mixed with the same amount of vanillin (8%, w/v) and twice the amount of sulphuric acid (72%, w/v). The mixture was incubated at 60 °C for 10 min, followed by cooling in an ice-water bath for 15 min. Absorbance was read at 535 nm. The total saponin content was expressed as quillaja equivalents (mg QAE/g).

Phenolic compound analyses

Phenolic compounds of *C. aucheri* were analyzed by RP-HPLC (Shimadzu, Japan) as described by Caponio et al. [16] with some modifications. Separation was performed at 30 °C by using a reversed-phase column (Agilent Eclipse XDB C-18, 250 mm × 4.6 mm, 5 μm) using the mixture of two solvents (A: the acetic acid solution 3% and B: methanol) as a mobile phase. Gradient conditions were particularized at a flow rate of 0.8 mL/min as follows: 93% A + 7% B for 0–20 min, 72% A + 28% B in 20–28 min, 75% A + 25% B in 28–35 min, 70% A + 30% B in 35–60 min, 67% A + 33% B in 60–62 min, 58% A + 42% B in 62–70 min, 50% A + 50% B in 70–73 min, 30% A + 70% B in 73–75 min, 20% A + 80% B in 75–80 min, 0% A + 100% B in 80–81 min, 93% A + 7% B in 81–90 min. Phenolic compounds in the methanolic extract of *C. aucheri* were expressed as μg/g extract and analyzed with a diode-array detector (DAD) at 280 nm (for the phenolic acids) and 320 and 360 nm (for the flavones and flavonols, respectively). The quantitative analysis was made by comparing the standards used (gallic acid, protocatechuic acid, catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, naringin, hesperidin, rosmarinic acid, eriodictyol, cinnamic acid, quercetin, luteolin, kaempferol, apigenin). The identification of each target compound was based on a

combination of retention time and spectral matching. The HPLC method was validated as to linearity, accuracy, and sensitivity. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using signal to noise ratio method.

Antioxidant activity assays

Ferric reducing antioxidant power (FRAP) assay was applied as described by Zengin et al. [17] with some modifications. Extract solutions were added to the FRAP reagent, which was mixed in advance [acetate buffer – 0.3 M, TPTZ (2,4,6-tripyridyl-s-triazine) – 10 mM, $FeCI_3$ – 20 mM]. After measuring the absorbances at 593 nm, FRAP activity was expressed as Trolox (mg TEs/g extract) equivalents.

Total antioxidant capacity (Phosphomolybdenum method)

The phosphomolybdenum method was used to evaluate the total antioxidant capacity of the extracts. Briefly, different extract solutions were mixed with the reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM $(NH_4)_2MoO_4$) and incubated for 90 min at 95 °C. The absorbance values were determined at 695 nm wavelength [18]. Total antioxidant capacity was expressed as Trolox equivalent (mmoL TE/g extract).

Metal chelating activity

Extract solutions at different concentrations were added to $FeCl_2$ (0.05 mL, 2 mM). The reaction that started directly after adding 5 mM of ferrozine was measured at 562 nm after 10 min left at room temperature. Metal chelating activity was expressed as ethylenediaminetetraacetic acid (EDTA) equivalent (mg EDTAE/g extract) [17].

Nitric oxide (NO) scavenging activity

NO was produced from sodium nitroprusside (SNP), which was measured, as described by Balakrishnan et al. [19], by using the Griess reaction. The mixture containing SNP (5 mM) in phosphate-buffered saline (PBS) (pH 7.3) was prepared with the extracts in PBS at different concentrations and incubated for 3 hrs at 25 °C. The absorbance value was determined at 546 nm wavelength. Ascorbic acid was used as a positive control. The results were indicated as IC_{50}.

ABTS [*2,2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)*] *radical scavenging activity*

The scavenging activity towards ABTS radical was analyzed as described by Re et al. [20] with some modifications. Freshly prepared and diluted ABTS solution was mixed with the various solvent extracts of *C. aucheri*, and the absorbances were read after 30 min at 734 nm. The results were indicated as IC_{50}.

Cell culture and cell antiproliferation capacity

HeLa (as human cervix adenocarcinoma cell line) and H1299 (as non-small cell lung adenocarcinoma cell line) cells were used and cultured in RPMI 1640 medium in a CO_2 incubator. After being seeded into 96-well plates (2×10^3 cells/well) and a 24 hrs incubation, the medium was removed from the well, leaving the adherent cells. The cells were applied with extract for 24, 48, and 72 hrs in the range of 3.125–100 µg/mL. After the time was up, cytotoxicity was determined using the CellTiter-Glo assay. Viability was calculated using the background-corrected absorbance as follows: Viability (%) = Abs of experiment well / Abs of control well x 100.

Statistical analysis

Statistical analysis was performed using the software SPSS version 22.0 program. Statistical significance was determined using the one-way ANOVA. Multiple group comparisons were analyzed with Tukey's multiple comparison test. Data were expressed as mean ± standard deviation (SD) and *p*-value of < 0.05 was considered statistically significant.

Results

Total bioactive compounds

C. aucheri collected from its single location in Turkey was extracted using methanol, acetone, and petroleum benzine. Then, the total phenolic, flavonoid, and saponin contents (TPC, TFC, and TSC, respectively) of these extracts were investigated with spectrophotometric methods, and the results are presented in Table 1. According to the obtained

Table 1

Total phenolic, flavonoid, and saponin contents of *Convolvulus aucheri* extracts (mean ± SD)

Solvent	TPC[x]	TFC[y]	TSC[z]
Petroleum benzine	22.06 ± 0.05[c]	05.04 ± 0.01[c]	11.08 ± 0.03[c]
Acetone	40.05 ± 0.07[b]	10.35 ± 0.02[b]	37.12 ± 0.06[b]
Methanol	87.64 ± 1.12[a]	51.76 ± 0.06[a]	71.30 ± 1.06[a]

TPC – [x]total phenolic content expressed as gallic acid equivalents (mg GAEs/g); TFC – [y]total flavonoid content expressed as quercetin equivalents (mg QEs/g); TSC – [z]total saponin content expressed as quillaja equivalents (mg QAEs/g); SD – standard deviation.
***p* < 0.05 compared with: [a] – petroleum benzine and acetone; [b] – petroleum benzine and methanol; [c] – acetone and methanol.**

data, the total phenolic content (87.64 mg GAEs/g), total flavonoid content (51.76 mg QEs/g), and total saponin content (71.30 mg QAEs/g) were detected to be at the highest in the methanol extract of the plant.

HPLC analysis

Because the methanol extract had higher total phenolic content than the others, it was used in RP-HPLC analysis by using 24 standard compounds (Figure 1) in order to elucidate the phenolic profile of *C. aucheri*. Protocatechuic acid, (+)-catechin, chlorogenic acid, caffeic acid, ferulic acid, rutin, rosmarinic acid, eriodictyol, quercetin, and kaempferol were detected in the extract in varying amounts (Figure 2). Chlorogenic acid (22,690 μg/g) was found as the main component of the extract in the present study, followed by (+)-catechin (8,630 μg/g), rosmarinic acid (4,370 μg/g), and rutin (1,810 μg/g) (Table 2).

Antioxidant activity

The outcomes of free radical scavenging assays (ABTS and NO), phosphomolybdenum, metal chelating, and ferric reducing power assays are presented in Table 3. In both ABTS and NO assays, there were significant differences ($p < 0.05$) between the various extracts (methanol, acetone, and petroleum benzine). In the current study, the highest scavenging activity values obtained from the NO (76.03

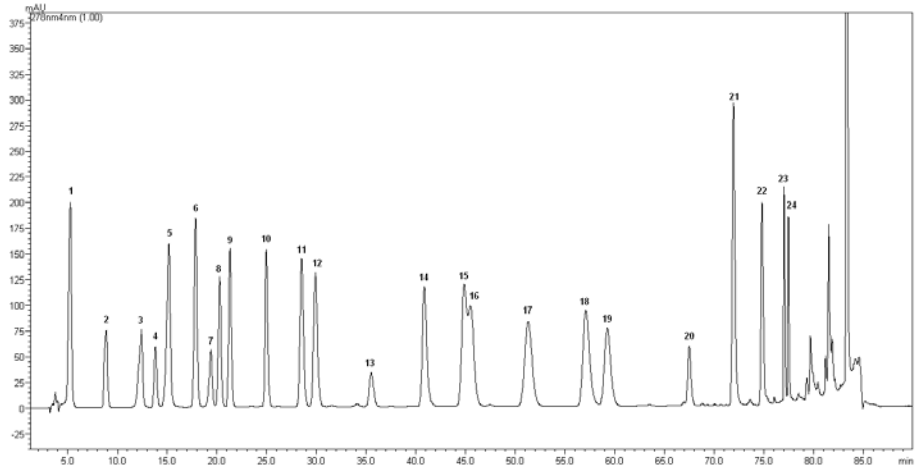


Fig. 1 – High-performance lipid chromatography (HPLC) chromatogram of standards: 1 – gallic acid; 2 – protocatechuic acid; 3 – catechin; 4 – *p*-hydroxy benzoic acid; 5 – chlorogenic acid; 6 – caffeic acid; 7 – epicatechin; 8 – syringic acid; 9 – vanilin; 10 – *p*-coumaric acid; 11 – ferulic acid; 12 – sinapinic acid; 13 – benzoic acid; 14 – *o*-coumaric acid; 15 – rutin; 16 – naringin; 17 – hesperidin; 18 – rosmarinic acid; 19 – eriodictiol; 20 – cinnamic acid; 21 – quercetin; 22 – luteolin; 23 – kaempferol; 24 – apigenin.

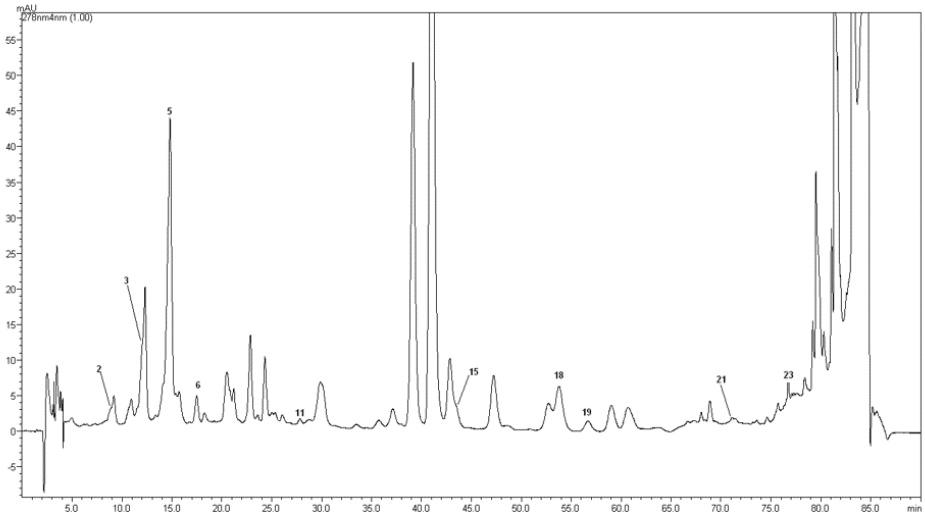


Fig. 2 – High-performance lipid chromatography (HPLC) chromatogram of *Convolvulus aucheri* extract where: 2 – protocatechuic acid; 3 – catechin; 5 – chlorogenic acid; 6 – caffeic acid; 11 – ferulic acid; 15 – rutin; 18 – rosmarinic acid; 19 – eriodictiol; 21 – quercetin; 23 – kaempferol.

Table 2

Phenolic compounds of *Convolvulus aucheri* and high-performance lipid chromatography (HPLC) validation results

Identified phenolic compounds	RT (min)	UV_{max} (nm)	LOD (μg/mL)	LOQ (μg/mL)	Linearity range (μg/mL)	μg/g extract (mean ± SD)
Protocatechuic acid	9.13	280	0.031	0.102	0.263–207.11	850.0 ± 6.0
(+)-Catechin	12.22	280	0.020	0.096	0.258–205.45	8,630.0 ± 176.0
Chlorogenic acid	15.00	320	0.010	0.362	0.250–203.57	22,690.0 ± 490.0
Caffeic acid	17.32	280	0.019	0.043	0.260–209.20	780.0 ± 5.3
Ferulic acid	28.11	320	0.021	0.031	0.275–255.17	310.0 ± 3.5
Rutin	43.23	360	0.050	0.166	0.411–386.33	1,810.0 ± 24.0
Rosmarinic acid	54.00	320	0.016	0.041	0.302–295.02	4,370.0 ± 96.0
Eriodictyol	56.71	280	0.025	0.095	0.256–233.14	550.0 ± 4.0
Quercetin	71.21	320	0.048	0.159	0.432–398.32	290.0 ± 3.0
Kaempferol	77.12	320	0.030	0.106	0.288–261.53	780.0 ± 5.0

RT – retention time; LOD – limit of detection; LOQ – limit of quantification; SD – standard deviation.

Table 3

Antioxidant activities of *Convolvulus aucheri* extracts (mean ± SD)

Solvent	ABTS (IC_{50} μg/mL)	NO (IC_{50} μg/mL)	FRAP assay (mg TEs/g)	Phosphomolyb-denum assay (mmoL TEs/g)	Metal chelating activity (mg EDTAEs/g)
Petroleum benzine	113.65 ± 2.08[a]	128.02 ± 2.77[a]	41.33 ± 0.18[a]	0.33 ± 0.01[a]	4.97 ± 0.01[a]
Acetone	75.13 ± 1.01[b]	94.13 ± 1.72[b]	59.30 ± 0.06[b]	1.38 ± 0.05[b]	16.41 ± 0.03[b]
Methanol	45.64 ± 0.09[c]	76.03 ± 1.05[c]	80.41 ± 1.80[c]	2.45 ± 0.07[c]	25.35 ± 0.06[c]
Ascorbic acid	6.53 ± 0.01[d]	15.06 ± 0.03[d]	nt	nt	nt

ABTS – 2,2 azino-bis (3-ethilbenzothiazoline-6 sulfonic acid); NO – nitric oxide; FRAP – ferric reducing antioxidant power; TEs – Trolox equivalents; EDTA – ethylenediaminetetraacetic acid; EDTAEs – EDTA equivalents; nt – not tested; SD – standard deviation.
***p* < 0.05 compared with: [a] – acetone, methanol, and ascorbic acid; [b] – petroleum benzine, methanol, and ascorbic acid; [c] – petroleum benzine, acetone, and ascorbic acid; [d] – petroleum benzine, acetone, and methanol.**

μg/mL) and ABTS (45.64 μg/mL) assays were detected by methanol extracts. The total antioxidant capacity of extracts was examined by the phosphomolybdenum method, which measures phenolic and nonphenolic compounds related to their reductive activity. The methanolic extracts of *C. aucheri* demonstrated the most powerful total antioxidant activity at 2.45 mmol TEs/g (Table 3) ($p < 0.05$). *C. aucheri* methanolic extracts showed the strongest FRAP activity with 80.41 mg TEs/g, whereas the lowest activity was found in petroleum benzine extracts at 41.33 mg TEs/g. Similar to ABTS and NO assays, *C. aucheri* demonstrated great FRAP activity in its methanolic extract. In conformity with outcomes of other antioxidant tests, effective chelation power was again determined in the methanol extract with 25.35 mg EDTAEs/g.

Cytotoxic activity

In order to evaluate the cytotoxic activity of the *C. aucheri*, the methanol extract was used due to its higher total bioactive compounds and antioxidant activity than other extracts. The cytotoxic activity of the methanolic extracts on the growth of HeLa and H1299 cells was determined using the CellTiter-Glo assay. A decrease in viability in cancer cell lines was observed in a concentration-dependent manner ($p < 0.05$) (Figures 3 and 4). The IC_{50} values (μg/mL) that cause

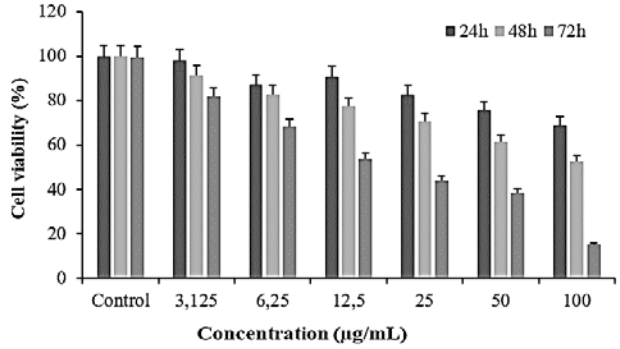


Fig. 3 – Concentration and time-dependent inhibitory effects of *Convolvulus aucheri* extract on cell viability in HeLa cells. Data are presented as mean ± standard deviation.

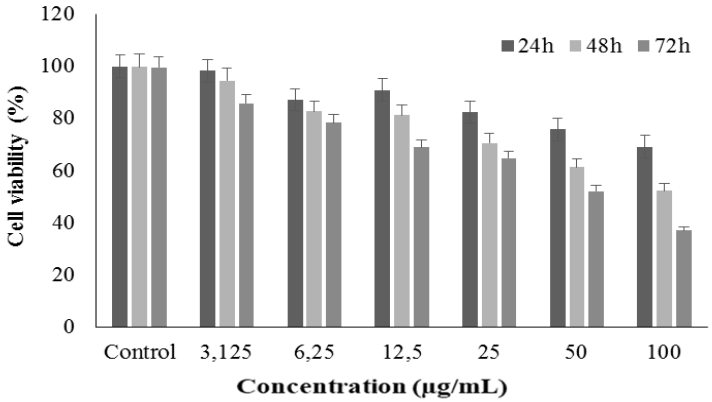


Fig. 4 – Concentration and time-dependent inhibitory effects of *Convolvulus aucheri* extract on cell viability in H1299 cells. Data are presented as mean ± standard deviation.

50% cell death were calculated at 14.22 µg/mL and 64.45 µg/mL on HeLa and H1299 cells, respectively.

The total phenolic content (87.64 mg GAEs/g), total flavonoid content (51.76 mg QEs/g), and total saponin content (71.30 mg QAEs/g) were detected to be at the highest in the methanol extract of the plant.

Discussion

Thanks to many beneficial effects of the secondary metabolites, the plants have been the center of interest for many years [21]. Alkaloids, flavonoids, coumarins, sterols, saponins, and tannins have been isolated from plants of the genus *Convolvulus* L. [22, 23]. The total phenolic and flavonoid content of *C. galaticus* was reported earlier at 84.689 mg GAEs/g and 48.760 mg CEs/g, respectively [24]. TPC of *C. hystrix* was reported at 14.6 mg GAEs/g for ethyl acetate extract [25]. Elzaawely and Tawata [10] found the total phenolic and flavonoid content of *C. arvensis* leaves at 244.6 mg GAE/g and 174.4 mg RE/g, respectively. According to these results, *C. aucheri* methanolic extract used in the study had more abundant (87.64 mg GAEs/g) total phenolic contents than *C. galaticus* and *C. hystrix*. Despite having various amounts of bioactive compounds as a result of using different taxa, solvents, and growing conditions, it would not be surprising to say that most of these *Convolvulus* taxa could be a significant source of phenolic compounds.

The phenolic profile of *C. galaticus* methanolic extracts was obtained by using a liquid chromatography-tandem mass spectrometry analysis [24]. The authors reported that 7 phenolic and flavonoid compounds were detected in the extracts. Methanol extracts of *C. galaticus* contained from the lowest to the highest amount of epigallocatechin (0.094 µg/g), *p*-coumaric acid (4.01 µg/g), vanillic acid (6.264 µg/g), kaempferol (14.832 µg/g), coumarin (15.382 µg/g), caffeic acid (157.432 µg/g), and rutin (286.9 µg/g). Similarly, *C. arvensis* methanol extract demonstrated an abundant amount of phenolic compounds, including *p*-coumaric acid (54.50 µg/g), *p*-hydroxybenzoic acid (47.82 µg/g), and syringic acid (22.50 µg/g) [26]. The phenolic and flavonoid compounds of these extracts mentioned above are quite different from those found in the current study. The amount of caffeic acid (780.0 µg/g), kaempferol (780.0 µg/g), and rutin (1,810.0 µg/g) was remarkably higher in *C. aucheri* methanolic extract than in methanolic extract of *C. galaticus*. Chlorogenic acid (22,690 µg/g) was the major phenolic in *C. aucheri.* Other major components in the extract were (+)-catechin (8,630 µg/g), rosmarinic acid (4,370 µg/g), and rutin (1,810 µg/g). The major components exhibit a broad spectrum of biological activities, including antioxidant, anticancer, and antimicrobial [27–29]. It has been demonstrated that the amounts of chlorogenic acid found in the extract are tightly correlated to metal chelating activity [30]. In accordance with the literature, we can conclude that the major polyphenolic compounds such as chlorogenic acid, (+) catechin, rosmarinic acid, rutin, protocatechuic acid, and kaempferol present within the methanolic extract of *C. aucheri* are responsible for its antioxidant activity. The results showed that major phytochemicals in *C. aucheri* were polar compounds extracted by methanol, which were determined to have the most potent antioxidant activity, including phenolics and saponins.

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce reactive oxygen species (ROS). Some antioxidants stop or eliminate the side effects of ROS and prevent some disorders, such as cancer, cardiovascular diseases, diabetes, infections, and ischemia [31]. The use of only one method does not reflect the antioxidant activity of plant extracts due to the complicated structure of bioactive secondary metabolites. Hence, chiefly five methods were used in order to detect the antioxidant activity of *C. aucheri* with different solvents. Health-damaging lipid peroxidation may be induced by ferrous ions, which have a significant mission as catalysts in the production of OH radicals. Therefore, the metal ion chelating ability of some phytochemicals is an important activity in eliminating the free radicals. The chelating capacities of the extracts were estimated by Ferrozine assay. The obtained results showed that the methanolic extract has the highest capacity to chelate ferrous ions. Al-Rifai et al. [32] determined the antibacterial and antioxidant activities of the ethanol extracts of two *Convolvulus* species namely, *C.*

austroaegyptiacus and *C. pilosellifolius*, and reported that the DPPH radical scavenging activity of *C. austroaegyptiacus* (IC_{50}: 21.81 μg/mL) was lower than that of *C. pilosellifolius* (IC_{50}: 17.62 μg/mL). Thakral et al. [33] reported that the IC_{50} values obtained from the DPPH and NO assays in *C. arvensis* methanolic extracts were determined at 131.03 and 130.12 μg/mL, respectively. The researchers found that phenolic compounds such as phenolic acids, flavonoids, and tannins, which are found in *C. arvensis*, could be the responsible compounds for antioxidant activity [33, 34]. In the current research, the antioxidant activity of the studied extracts exhibited a satisfactory correlation with their total phenolic, flavonoid, and saponin contents. Our results display that these bioactive compounds are consistent with the assessed antioxidant activity tests. The antioxidant activities of the extracts changed in the same order as the total bioactive compounds (methanol>acetone>petroleum benzine). In this context, the bioactive compounds could be considered major contributors to the antioxidant effect of *C. aucheri* extracts.

Cervical cancer is the third deadliest cancer for women in the emerging countries [35]. Plant origin products show hopeful resources of antitumor substances with lower adverse effects as compared to synthetic medications [36]. In previous studies, researchers demonstrated that different solvent extracts of *C. arvensis* had superior cytotoxic potential on lymphoblastic leukemia, Jurkat cells, rhabdomyosarcoma tumor cells, and HeLa cells [9, 37, 38]. Kaur and Kalia [39] reported that *C. arvensis* ethanolic extract had prominent inhibition on Colo-205 and IMR-32 cell viability by 73% and 85%, respectively. In another study, researchers showed that the *C. arvensis* extract could be regarded as a hopeful anticancer agent, with over 50% prevention of cancer progress [40]. As cancer cells grow, they secrete chemicals that induce new blood vessel growth, which is called angiogenesis. The extracts of *C. arvensis* were reported to have the capability to block angiogenesis in cancer [41]. In addition, the stimulating effect of this plant on the immune system was also revealed [42]. Polyphenols and saponins have been proved as anticancer agents [43, 44]. It is reported that the anticancer effect of *C. arvensis* may be caused by these phytochemicals. In terms of the presence of phenolics in the *C. aucheri*, our results are in good agreement with the literature. In this study, kaempferol in the extract was determined at 780.0 μg/g. It is known that kaempferol, which is a flavonoid, exhibits antitumor properties through the induction of G2/M phase cell cycle arrest and apoptosis on HeLa cells [45].

Conclusion

For the first time, this research notifies the antioxidant, antiproliferative potentials, and HPLC profile of *C. aucheri*. The methanol extract of *C. aucheri* was determined to have different phenolic compounds as identified by HPLC. It can be proposed that the obtained biological activity of the *C. aucheri* extract can be ascribed to the existence of these phenolic compounds. Our results showed that *C. aucheri* could be accepted as a novel and alternative natural antioxidant and antitumor source. For this reason, this plant may shed light on the design of the new drug or food additive formulations. On the other hand, such studies are valuable from the aspect of revealing the contents of traditional plants. Although this is the first study carried out on this plant, further *in-vitro* and *in-vivo* studies are necessary in order to better understand its potential.

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Conflict of interest

The authors declare no conflict of interest.

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