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# HPLC Analysis of Phenolic Compounds from *Gypsophila aucheri* Boiss. and Investigation of Antioxidant and Cytotoxic Activity of *Gypsophila aucheri* Boiss. exracts

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**Abctract**

This study was designed to investigate the antioxidant and antiproliferative activities of *Gypsophila aucheri* Boiss. extracts as well as phenolic content by RP-HPLC technique. Antioxidant activity potentials of the extracts were evaluated by four different methods namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity tests, cupric ion reducing antioxidant capacity (CUPRAC) method, and metal chelating assay. Antiproliferative activities of the extracts were tested against breast carcinoma (MCF-7), colorectal carcinoma (HT-29), and hepatocellular carcinoma (HepG2) cells. RP-HPLC analysis revealed the presence of eight phenolic compounds in eighteen phenolics scanned. Antioxidant activity results showed that methanol extract of *Gypsophila aucheri* displayed more pronounced antioxidant activity than water extract according to DPPH and ABTS tests, whereas water extract was superior than methanol extract for CUPRAC and metal chelating activity tests. Cytotoxicity tests demostrated the promising antiproliferative activity of methanol extract against all the tested cancer cell lines with its lower $IC_{50}$ values. However, both extracts were observed to display moderate biological activity compared to positive controls. The obtained data suggest that *Gypsophila aucheri* could be evaluated as a promising source for food and nutraceutical industries due to its striking antioxidant and moderate antiproliferative potentials together with high phytochemical profile.

**Keywords:** Antiproliferative activity, Antioxidant activity, Cell Culture, *Gypsophila aucheri* Boiss., Phenolic compounds

## *Gypsophila aucheri* Boiss. Bitkisinin Fenolik İçeriğinin HPLC Analizi ve *Gypsophila aucheri* Boiss. Ekstrelerinin Antioksidan ve Sitotoksik Aktivitelerinin Araştırılması

**Öz**

Bu çalışma, Gypsophila aucheri Boiss. ekstrelerinin antioksidan ve antiproliferatif aktivitelerinin yanı sıra RP-HPLC tekniği ile fenolik içeriğini araştırmak için tasarlanmıştır. Ekstrelerin antioksidan aktiviteleri 1,1-difenil-2-picrilhidrazil (DPPH) ve 2,2′-azinobis- (3-etilbenzotiazolin-6-sülfonik asit) (ABTS) radikallerini giderme, Bakır(II) iyonlarını indirgeme (KUPRAK) ve $Fe^{2+}$ iyonlarını şelatlama kapasiteleri üzerinden değerlendirildi. Antiproliferatif aktivite için, ekstrelerin MCF-7 (insan meme kanseri), HT-29 (kolon kanseri) ve HepG2 (karaciğer kanseri) hücrelerine karşı test edildi. Antioksidan aktivite sonuçları, Gypsophila aucheri metanol ekstresinin DPPH ve ABTS testlerine göre su ekstraktından daha güçlü antioksidan aktivite gösterdiğini, su ekstresinin ise KUPRAK ve metal şelatlama aktivitesi testlerinde metanol ekstresinden daha üstün olduğu gözlendi. Sitotoksisite testleri sonucunda, metanol ekstresinin su ekstresine kıyasla daha düşük IC50 değerleriyle test edilen tüm kanser hücre hatlarına karşı daha güçlü antiproliferatif aktivite sergilediğini gösterdi. Bununla birlikte, her iki ekstrenin de pozitif kontrollere kıyasla orta düzeyde biyolojik aktivite sergilediği saptandı. Elde edilen veriler, Gypsophila aucheri Boiss.'in yüksek fitokimyasal içeriğiyle birlikte çarpıcı antioksidan ve orta antiproliferatif potansiyelleri nedeniyle bu bitki kaynağının gıda ve nutrasötik endüstri için umut verici bir kaynak olarak değerlendirilebileceğini göstermektedir.

**Keywords:**Antiproliferatif aktivite, Antioksidan aktivite, Hücre kültürü, *Gypsophila aucheri* Boiss., Fenolik bileşikler

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## 1. Introduction

Medicinal plants serving as a rich source of pharmacologically active phytochemicals have been gained much more attention due to their many uses in a diverse array of purposes such as medicine, nutrition, flavorings, beverages, dyeing, repellents, cosmetics, charms and smoking (Kontogianni et al., 2013). There are many epidemiologic studies showing consumption of high phenolic content-diet is related with prevention of numerous biological disorders such as cardiovascular diseases and certain types of cancer (Altay and Bozoğlu, 2017; Jang et al., 2003; Van Dam et al., 2013). The action modes of the phenolic compounds might be attributed to their antioxidant behaviors, because they can stop or prevent the oxidation of many significant macromolecules in the cell (Farzaneh and Carvalho, 2015; Köksal et al., 2017b). Moreover, they prevent or slow down the progression of many chronic diseases caused by free radical-induced oxidative stress (Köksal et al., 2017a). Besides, antioxidants have commonly been used in food and pharmaceutical industries to contribute to protection against oxidative degradation of foods (Ngo et al., 2011). In addition, phenolic compounds from plants possess anti-inflammatory, anti-bacterial, anti-proliferative, anti-mutagenic, anti-carcinogenic activities, substantially (dos Santos et al., 2017). However, many plants, which are used as food or traditional medicine may become mutagenic, cytotoxic or genotoxic for healthy cells, resulting from the long-term usage (Poyraz et al., 2017). Therefore, cytotoxicity tests are very useful to determine the dose of concentration to be used, and also these tests may contribute to more detailed studies in order to obtain significant information on some parameters, including genotoxicity, induce of mutations and programmed cell death (Guérard et al., 2015). Many studies have been carried out on natural sources to unravel the phenolic components possessing high antioxidant potential with low cytotoxicity for healthy cells, while high cytotoxicity for cancerous cells (Poyraz et al., 2017). Because of diverse biological roles, the identification and quantification of phytochemicals in different plant species are very essential (Gülçin, 2011). The family of Caryophyllaceae that is mainly distributed in the northern and southern hemisphere as well as Mediterranean region is represented by 80 genera with 2100 species (Korkmaz and Özçelik, 2013). The genus Gypsophila L. having 126 species worldwide is mainly distributed in the Irano-Turanian and Mediterranean regions and it is the third biggest genus of Caryophyllaceae family in Turkey, possessing 55 species in the country and represented by 58 taxa and 33 of them are endemic (Korkmaz and Özçelik, 2011). Gypsophila species have been widely used in folk medicine throughout the world due to its diverse medicinal purposes such as spermicidal, hypocholesterolaemic, anti-inflammatory and antiviral activities (Primorac et al., 1985), antioxidant activity (Vitcheva et al., 2011), cytotoxic activity (Bai et al., 2007). They are also used to treat fever, consumptive disease, and infantile malnutrition syndrome (Yao et al., 2010). Additionally, some Gypsophila species are used in food industry in a wide array of purposes such as production of soaproot, ice-cream, liquor and herby cheese (Koyuncu et al., 2008), as well as adding to halva for obtain (Korkmaz and Özçelik, 2011). Moreover, because of their good sparkling properties and high saponin contents, they are used in various industrial applications such as soap, detergent and expectorant production (Korkmaz and Özçelik, 2011). Furthermore, the saponin sources of some Gypsophila species are used in gastronomy in Arabic countries (Yücekutlu and Bildacı, 2008). In the present study, we aimed to investigate the in vitro antioxidant and

antiproliferative potentials of methanol and water extracts of endemic plant species Gypsophila aucheri Boiss. as well as their phenolic contents. To our knowledge, this is the first study to reveal these properties of Gypsophila aucheri. Phenolic contents of the extracts were identified and quantified by reversed-phase high-performance liquid chromatography (RP-HPLC) analysis. The antioxidant capacity of the extracts were evaluated with four different antioxidant assays namely DPPH assay, ABTS assay, CUPRAC and metal chelating activity assay. Cytotoxic activity of the extracts were evaluated against three cancer cell lines; MCF-7, HT-29 and HepG2 cells.

## 2. Material ve Method

### *2.1. Plant materials and chemicals*

The endemic plant material Gypsophila aucheri was collected from rocky slopes of Üzümlü-Tercan, Erzincan, Turkey, on 25 June 2017, at an altitude of 1373 m. The samples were taxonomically identified by Dr. Mustafa Korkmaz. The voucher specimens were prepared according to the herbarium techniques, enumerated (4357) and preserved at the Laboratory of Plant Systematic, Department of Biological Sciences, Erzincan University, Erzincan, Turkey. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Calbiochem Co. (San Diego, CA). Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium persulfate, foline-ciocalteu phenol reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium hydroxide, ethanol, and methanol were purchased from Sigma-Aldrich. All phenolic standard compounds and solvents being HPLC grade were also obtained from both Sigma-Aldrich and Merck (Darmstadt, Germany).

### *2.2. Sample preparation*

Gypsophila aucheri plants were extracted and analyzed shortly after the collection step. Briefly, the aerial parts of Gypsophila aucheri, protected from direct sun light were dried at room temperature. Briefly, the aerial parts the samples were mixed with 250 mL of extraction solvents (methanol and water) in a ration of 1:5 (w:v), and then left to shaken over night. This process was applied three times. In order to get ultra-dry powders, the extracts were filtered through Whatman No. 4 filter paper, and then concentrated under vacuum with rotary evaporator (Heidolph, Germany) at 40 °C, and then lyophilised using a Scanvac Cool Safe™ freeze-dryer (Cool-Safe 55, Lynge, Denmark). The extracted powder was weighed and stored at -20°C in brown bottle until use.

### *2.3. Total phenolic and flavonoid content*

The total phenolic amounts of Gypsophila aucheri extracts were determined by Folin-Ciocalteu method of (Singleton and Rossi, 1965), which is modified by Altay et al. (2017). Briefly, 100 µL of Folin reagent (20% ) was mixed with 20 µL of the extract at a certain concentration into the 96 well plate. After that, 80 µL of sodium carbonate solution (10%) was added and incubated for 30 minutes at room temperature. After incubation time, the absorbance values were recorded at 750 nm by using Elisa microplate reader. Results were reported in terms of gallic acid equivalents (GAE). Each test was performed in three replicates and the results were expressed as mean ± standard deviation (SD). Total flavonoid content of Gypsophila aucheri extracts were evaluated by using Aluminium chloride colorimetric method (Zhishen et al., 1999), which is modified by Altay et al. (2017). Briefly, 20 µL of the extracts at a certain concentration was mixed with 6 µL of sodium nitrite (5%) into the 96 well plate that contain 80 µL of distilled

water and incubated for 5 min. After incubation time, 6 µL of aluminium chloride solution (10 %) was added and incubated another 6 min. Ahter that 40 µL of sodium hydroxide (1 M) was added and the final volume was completed to 200 µL with distilled water. The absorbance of the reaction mixture was measured at 510 nm by using Elisa microplate reader. The results were reported in terms of quercetin equivalents (QE). Each experiment was run in three replicates and the results were expressed as mean ± standard deviation (SD).

### *2.4. RP-HPLC analysis*

RP-HPLC analysis were performed by using a Dionex UltiMate 3000 HPLC system, which is equipped with UltiMate 3000 Pump, UltiMate 3000 Autosampler Column Compartment, UltiMate 3000 Photodiode Array Detector and Chromeleon software. The separation was carried out by using an Agilent Zorbax SB-C18 (250mm x 4,6mm x 5µm) with a guard column packed with the same material. The column was maintained at 30 oC throughout the analysis, and 280 nm was selected as the wavelength for UV detection. Gradient elution was carried out at a flow rate of 1.0 mL/min at 30°C. The mobile phase consisted of (A) methanol:water [50% (v/v)] and (B) water:acetic acid [98:2 by volume]) using a gradient elution as follows: 0–3 min, 0% B; next 3-5 min, 8% B; next 5-57 min, linear change from A-B (92:8) to A-B (28:72), then back to 100% A at 57-60 min. 0.45µm membrane filter (Millipore, Milford, MA) was used for all samples, and 20 µL of the filtrated extracts and standards were injected into HPLC for analysis. Three HPLC replicate injections were performed for plant extracts and each standard phenolic compound.

### *2.5. Antioxidant activity studies*

#### *2.5.1. DPPH radical scavenging activity*

DPPH assay was performed by using the method of (Blois, 1958). Briefly, 140 µL of DPPH solution (0.05 mg/mL) was mixed with 10 µL of each extract into the 96 well plate and incubated upto 30 min. After the incubation time, the decrease of absorbance of the reaction mixture was monitored at 517 by using Elisa microplate reader. α-tocopherol, trolox, BHA and BHT were employed as reference control. According to the results, RSA% vs final concentrations of the extracts were plotted and $IC_{50}$ (50% inhibitory concentration) values were calculated. All measurements were performed three times.

#### *2.5.2. ABTS radical scavenging activity*

ABTS assay was carried out according to the method of (Re et al., 1999). Briefly, 2.5 µL of each extract solution at a certain concentration was mixed with 250 µL of ABTS radical solution into the 96 well plate and the absorbance of the reaction mixture was monitored at 734 nm using Elisa microplate reader. After the initial mixing of the reactants, time was recorded in every minute from 1st to 6th. The results were expressed in terms of $IC_{50}$ value (50% inhibitory concentration). All measurements were performed three times.

#### *2.5.3. CUPRAC assay*

Cupric ions' ($Cu^{2+}$) reducing capacities of Gypsophila aucheri extracts were evaluated by using CUPRAC method (Apak et al., 2008). Briefly, the tested extract solutions (10–30 µg/mL) were added into the tested tubes containing 0.25 mL of CH3COONH4 buffer solution (1.0 M), 0.25 mL of ethanolic neocuproine solution ($7.5 \times 10^{-3}$ M) and 0.25 mL of $CuCl_2$ solution (0.01 M) and the final volumes were completed to 2 mL with distilled water and incubated for 30 min at

room temperature. After the incubation time, the absorbances of reaction mixtures were recorded at 450 nm. Increased absorbance was considered as increased reducing capacity.

### *2.5.4. Determination of metal chelating activity*

Ferrious ions ($Fe^{+2}$) chelating activity of Gypsophila aucheri extracts were measured by using Dinis method (Dinis et al., 1994). Briefly, 50 µL of the extract solutions (0.5 to 4 mg/mL) were added into the 96 well plate, containing distilled water (185 µL), and FeCl2 (5 µL of 2 mM) solution and incubated 5 min. After incubation time, the reaction was initiated by adding 10 µL of 5 mM ferrozine solution into each well. After the incubation time (10 min.) at room temperature, the absorbance of the reaction mixture was recorded at 562 nm. EDTA was used as the chelating standard. The results were expressed in terms of $IC_{50}$ value. All measurements were performed three times.

### *2.6. Antiproliferative activity assay*

The cell lines; HT-29, HepG2, and MCF-7 were obtained from the ATCC (American Type Culture Collection, LGC Promochem, UK). MCF-7 and HepG2 cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 1% L-Glutamine, 10% fetal bovine serum, 1% penicillin-streptomycin (Pen Strep) solution and 1% Na-pyruvate. HT-29 cells were grown in a medium McCoys's 5A containing 10% fetal bovine serum, 1% L- Glutamine and 1% penicillin-streptomycin solution. Cultures were incubated at 37°C with 5% carbon dioxide (CO2) and 95% humidity in incubator (NUVE, Turkey). Studies were performed in Metisafe Class II Safety Cabinet. The cytotoxic potentials of the extracts on the cancer and healthy cells were evaluated by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide (XTT) assay, which is based on the extracellular reduction of tetrazolium salt XTT by NADH produced in the mitochondria via trans-plasma membrane electron transport and an electron mediator (Trendowski et al., 2014). The assay is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT to orange colored compounds of formazan. The dye formed is water soluble and the dye intensity can be read at a given wavelength with a spectrophotometer. The intensity of the dye is proportional to the number of metabolic active cells. The cells were seeded at a concentration of 1x105 cells/mL (100 µL) into 96-well culture plates and allowed to adhere overnight at 37 °C. After 24 h, cells were treated with the test compounds at different concentrations, in triplicate, or with a solvent control (0.5 % DMSO) in complete medium. After 48 h incubation, 50 µL of the XTT reaction solution that is prepared by adding 100 µL of activation solution to 5 mL of XTT reagent was added into each well and incubated for eight hours. After the incubation time, the plates were gently shaken and the absorbance were measured at 415 nm by Epoch Microplate Reader (BioTek, USA). 5-Fluorouracil (5-FU) was used as a standard reference drug.

### *2.7. Statistical analysis*

Statistical analyses were performed for evaluation of antioxidant and cytotoxic activity results by unpaired Student's t-test by using statistical program of GraphPad Prism 6 (GraphPad, La Jolla, CA) Software 7.0). All results were expressed as means with their standard deviation (SD). $p<0.05$ was taken as the minimum level of significance.

## 3. Result and Discussion

### *3.1. Phytochemical composition*

Many herbs and formulations are still used today for their therapeutic effects. Their diverse biological actions might be attributed to their rich phytochemical contents

(Hosseinzadeh et al., 2015). Therefore, it is highly significant to establish a direct correlation between the diverse biological activity potentials and phytochemical ingradients of the plants. In the present study, we first tried to determine the phytochemical composition of Gypsophila aucheri both qualitative and quantitative analyses. The amounts of total phenolics and flavonoids in methanol extract were determined as 120.79 mg GAE/g extract and 48.76 mg QE/ g extract, respectively, while those of water extract were 109.70 mg GAE/g extract and 34.97 mg QE/ g extract, respectively. These results shows that flavones constitute about half of the total phenolic content in both extracts. In the literature, it was reported the total phenolic content of Gypsophila pilulifera as 6.5 mg GAE/g extract (Yazici and Özmen, 2017). In another study, total phenolic contents of Gypsophila arrostii, Gypsophila pilulifera and Gypsophila simonii from Turkey were reported as 0.26, 0.54 and 15.15 mg GAE/g extract, respecticely (Arslan and Çelik, 2013). Compared to the literature, the superiority of Gypsophila aucheri in terms of phenolic content comes to the forefront. In parallel to the total phenolic and flavonoid contents, eighteen individual phenolic compounds were also screened by HPLC analysis, and qualitative and quantitative results with analytical parameters were tabulated in Table 1. As seen from the Table 1, methanol extract of *Gypsophila aucheri* include considerable amounts of gallic acid, 3,4-dihydroxybenzoic acid, catechin, vanillin, rutin and rosmarinic acid. The most abundant compound in the extract was 3,4-dihydroxybenzoic acid (0.72 μg/g dry plant), while the least one was vanillin (0.14 μg/g dry plant). On the other hand, water extract was found to contain considerable amounts of gallic acid, 2,3-dihydroxybenzoic acid, vanillin, syringic acid, p-coumaric acid, rutin and rosmarinic acid. p-coumaric acid and syringic acid were at the highest level (2.05 and 1.5 μg/g dry plant), respectively, whereas the amounts of other phenolics were very close to each other, changing between the range of 0.4 to 0.5 μg/g extract. Upon comparing two extracts considering Table 1, 3,4-dihydroxybenzoic acid and catechin was found only in methanol extract, while 2,3-dihydroxybenzoic acid, syringic acid and p-coumaric acid were detected only in water extract. This is not unexpected phenomenon since the polarity of the extraction solution affects the phenolic content of the plant extracts (Ammar et al., 2015; Do et al., 2014; Fu et al., 2016).

In the literature, there are many studies reporting that saponarin (apigenin-6- C-glucosyl-7-O-glucoside) is the main phenolic compound found in Gypsophila species (Arslan and Çelik, 2013; Chima et al., 2014; Vitcheva et al., 2011; Yazici and Özmen, 2017). Apart from the reports mentioned above, no other study is available in the literature regarding their individual phytochemical composition to compare that of Gypsophila aucheri by this study, we reported the first detailed quantitative data on Gypsophila aucheri.

### *3.2. Antioxidant activity*

There are many ways for evaluating the antioxidant potential of a source to have complete understanding of the mechanism of action. In the present study, the antioxidant potential of the methanol and water extracts of Gypsophila aucheri were evaluated by radical scavenging (DPPH and ABTS), reducing power (CUPRAC) and metal chelating ($Fe^{2+}$) assays. The results of radical scavenging and metal chelating assays were tabulated in Table 2, while CUPRAC results were shown in Figure 1.

**Table 1.** Phenolics and flavonoids of *Gypsophila aucheri* extracts (mean ± SD)[x].

| No. | Retention time(min) | Phenolics and flavonoids | Concentration (µg/g dry plant) | | Analytical characteristics | | |
|---|---|---|---|---|---|---|---|
| | | | Methanol | Water | $R^2$ | LOD (mg per L)[y] | LOQ (mg per L)[z] |
| **1** | 2.75 | Pyrogallol | nd[w] | nd | 0.998 | 0.003 | 0.01 |
| **2** | 2.97 | Gallic acid | 0.45±0.009[a] 0.52±0.02[a] | | 0.999 0.007 | | 0.002 |
| **3** | 5.60 | 3,4-Dihydroxybenzoic acid | 0.72±0.03 | nd | 0.999 0.001 | | 0.0003 |
| **4** | 8.92 | 2,3-Dihydroxybenzoic acid | nd 0.4±0.02 | | 0.997 0.001 | | 0.0003 |
| **5** | 9.37 | p-Hydroxybenzoic acid | nd | nd | 0.999 0.001 | | 0.003 |
| **6** | 11.94 | (+)-Catechin | 0.55±0.02 | nd | 0.997 0.009 | | 0.003 |
| **7** | 16.06 | Caffeic acid | nd | nd | 0.996 0.008 | | 0.002 |
| **8** | 16.88 | Chlorogenic acid | nd | nd | 0.998 0.004 | | 0.001 |
| **9** | 23.28 | Vanillin | 0.14±0.002[a] | 0.05±0.04[b] | 0.995 0.008 | | 0.002 |
| **10** | 23.97 | Syringic acid | nd 1.5±0.05 | | 0.996 0.009 | | 0.002 |
| **11** | 27.20 | (-)-Epicatechin | nd | nd | 0.999 0.0005 | | 0.0001 |
| **12** | 28.17 | p-Coumaric acid | nd 2.05±0.07 | | 0.998 0.001 | | 0.004 |
| **13** | 35.04 | Taxifolin | nd | nd | 0.997 0.009 | | 0.002 |
| **14** | 41.19 | Sinapic acid | nd | nd | 0.998 0.009 | | 0.002 |
| **15** | 49.35 | Resveratrol | nd | nd | 0.996 0.01 | | 0.003 |
| **16** | 50.07 | Rutin | 0.62±0.04[a] | 0.42±0.03[b] | 0.997 0.005 | | 0.001 |
| **17** | 51.89 | Rosmarinic acid | 0.19±0.03[a] | 0.43±0.01[b] | 0.998 0.005 | | 0.001 |
| **18** | 55.50 | Naringenin | nd | nd | 0.996 0.005 | | 0.001 |

[x]Data marked with different superscripts (a and b) within the same row indicate significant difference statistically ($p < 0.05$). [y]LOD, limit of detection. [z]LOQ, limit of quantification. [w]nd, not detected

**Table 2.** Radical scavenging activity, reducing power and metal chelating potentials of *Gypsophila aucheri* extracts (mean ± SD) [x].

| Test samples | Assays | | |
|---|---|---|---|
| | Radical Scavenging | | Metal Chelating |
| | DPPH $IC_{50}$ (µg/mL) | ABTS $IC_{50}$ (µg/mL) | $Fe^{2+}$ chelating $IC_{50}$ (µg/mL) |
| *Gypsophila aucheri* methanol extract | 426.2± 3.26 [a] | 107.2±1.37 [a] | 174.2±3.48 [a] |
| *Gypsophila aucheri* water extract | 822.5±4.12 [b] | 139.2±2.95 [b] | 114.6±2.42 [b] |
| [q]BHA | 8.2±0.52 [c] | 15.6±0.85 [c] | nt |
| [y]BHT | 21.6±1.02 [d] | 7.2±0.56 [d] | nt |
| Trolox | 18.8±0.75 [d] | 12.4±0.56 [c] | nt |
| α-Tocopherol | 28.4±0.45 [e] | 18.7±0.78 [c] | nt |
| [z]EDTA | nt | nt | 3.47±0.27 |

[x]Data marked with different superscripts (a, b, c, d and e ) within the same column indicate significant difference statistically ($p < 0.05$). [q]BHA, Butylated Hydroxyanisole. [y]BHT, Butylated hydroxytoluene. [z]EDTA, Ethylenediaminetetraacetic acid. nt, not tested.

DPPH and ABTS free radicals have been widely used to evaluate the radical scavenging ability of antioxidants (Zhao et al., 2006). Therefore, radical scavenging power potential of the extracts was examined by using two different test systems, namely DPPH and ABTS. As can be seen from the results presented in Table 2, methanol extract of Gypsophila aucheri showed higher DPPH and ABTS radical scavenging activity with its lower IC50 values (DPPH; 426.2 µg/mL and ABTS; 107.2 µg/mL) when compared to the water extract (DPPH; 822.5 µg/mL and ABTS; 139.2 µg/mL). However, both extracts displayed moderate radical scavenging activity compared to all of the standard antioxidants tested (Table 2). The results obtained show us that the phytochemicals, which scavenge DPPH and ABTS radicals are more abundant in the methanol extract, and it was observed a noticeable correlation between high radical scavenging activity and high amounts of total phenolic/flavonoid content.The findings are closely consistent with the literature. Serteser et al. (2009) reported a study, where five Gypsophila species were tested for their DPPH radical scavenging activities, showing the $IC_{50}$ values of the species change between 3.1 mg/mL to 3.6 mg/mL (Serteser et al., 2009). In another study, $IC_{50}$ values of different Gypsophila pilulifera extracts for DPPH radical scavenging activity were reported as 0.446 mg/mL and 4.56 mg/mL, respectively (Chima et al., 2014; Yazici and Özmen, 2017). Different antioxidant activities from different species can be expected due to differences in methodology and experimental conditions used in the different studies (Tohidi et al., 2017). Moreover, the presence of potential antioxidant compounds, such as vitamins, flavonoids, phenolic acids and sulphur compounds present in plants, of cource, influence the antioxidant activity of the extract (Embuscado, 2015; Skrovankova et al., 2015). Transition metals can stimulate lipid peroxidation by generating hydroxyl radicals via Fenton reaction and also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, and thus leading to persistence of the chain reaction of lipid peroxidation. Therefore, the evaluation of

antioxidant activities of the plant species on metal chelating capacities is indispensable. Antioxidant activity of the extracts, in this line, was screened by $Fe^{2+}$ chelating assay. In contrast to radical scavenging activity, metal chelating assay was resulted in the superiority of water extract with an lower $IC_{50}$ value of 114.6 µg/mL, compared to methanol extract (IC50;174.2 µg/mL). On the other hand, the metal chelating capacities of both extracts was below that of EDTA (Table 2). In the literature, $Fe^{2+}$ chelating capacities of five Gypsophila species was reported to varied between 17.2 and 24.3%. However, in this study, Gypsophila aucheri methanol and water extracts could able to exhibit high chelating activity up to 51 and 63% (Serteser et al., 2009). The CUPRAC assay is commonly used to screen the antioxidant capacity of plant extracts, because of the requirements of a small number of equipment, as well as fast and reproducible results (Tusevski et al., 2014). In order to examine the reducing power of the extracts, the $Cu^{2+}$ to $Cu^{+}$ reduction in the presence of the extracts was investigated. As shown in Figure 1, both extracts of Gypsophila aucheri displayed a good cupric ions ($Cu^{2+}$) reducing capacity. Even, water extract of Gypsophila aucheri showed higher reducing power than BHT and α-tocopherol at 10 µg/mL concentration, while methanol extract displayed only higher reducing power than BHT and similar to α-tocopherol. However, at high concentrations, both extracts exhibited moderate activity, compared to all standard antioxidants. In parallel to the metal chelating capacity, the reduction power of the water extract is at the forefront compared to the methanol extract. Regarding reducing power of Gypsophila aucheri, there are limited number of studies in the literature. One of them was Gypsophila bitlisensis, possessing moderate reducing power compered to the standard antioxidants trolox and tocopherol (Işık et al., 2015).

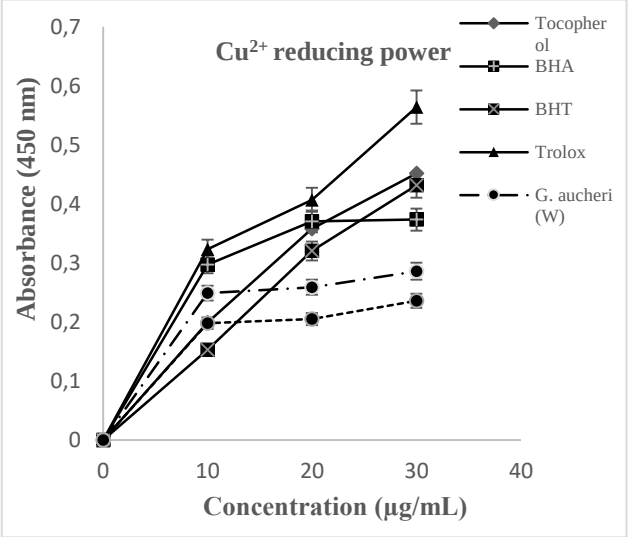



**Figure 1.** $Cu^{2+}$ reducing power of Gypsophila aucheri water (W) and methanol (M) extracts and standard antioxidants; Butylated Hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), trolox and α-tocopherol. Each point represents the average of three independent measurements, each done in triplicate, with the standard deviation of the mean.

All the antioxidant activity assays performed in this study indicate that a plant extract, exhibiting low antioxidant activity by a method could not be referred as a poor source of antioxidant since a plant extract includes phytochemicals with different functional groups and polarities as well as possessing different reaction mixture depending on the assay type (Tusevski et al., 2014). Detected phenolic compounds in the plant extracts, of course, are not responsible alone for the antioxidant activity. Contribution of other phytochemicals and their synergetic effects should also be taken into consideration.

### *3.3. Cytotoxic activity*

To evaluate the antiproliferative activities of the Gypsophila aucheri water and methanol extracts against human-derived cancer cell lines HT-29 (colorectal adenocarcinoma cell line), HepG2 (hepatocellular carcinoma cell line) and MCF-7 (breast adenocarcinoma cell line), cytotoxicity XTT assay was carried out. Growth inhibition percent was calculated

by comparing to a negative control growth after 48h incubation time and $IC_{50}$ values of the extracts and 5-Flourouracil (5-FU) used as a positive control were tabulated in Table 3.

**Table 3.** Antiproliferative activities of *Gypsophila aucheri* extracts against human-derived tumor cell lines.

| **Tested samples** | **$IC_{50}$ (mg/mL)[x]** | | |
|---|---|---|---|
| | HepG2 [a] | HT-29 [a] | MCF-7 [a] |
| Water extract | 0.79±0,032[b] | 0.57±0,17 [b] | 0.89±0,035 [b] |
| Methanol extract | 0.36±0,015[c] | 0.27±0,045 [c] | 0.51±0.052 [c] |
| [y]5-FU | 0,047±0,002[d] | 0,023±0,006 [d] | 0,018±0,004 [d] |

[a]Human tumor cell lines: HepG2, hepatocellular carcinoma; HT-29, colorectal adenocarcinoma; MCF7, breast carcinoma. [x]$IC_{50}$ (mg/mL): Data marked with different superscripts (b, c and d) within the same colum indicate significant difference statistically ($p < 0.05$). [y]5-FU: 5-Flourouracil (positive control).

The obtained results showed that the proliferation of three tested cell lines were significantly inhibited by Gypsophila aucheri extracts in a concentration-dependent manner depending on the cell types. The inhibition percent of the water and methanol extracts at maximum concentration (1 mg/mL) were 59.2% and 90.8% against HepG2 cells, 66.9% and 93.9% against HT-29 cells, and 56.9% and 93.2% against MCF-7 cells, respectively (Figure of cell percent inhibition was not shown). Whereas, the inhibitory ratios of 5-FU at 0.1 mg/mL against the three cell lines were 95.2%, 96.3%, and 93.1%, respectively (data not shown). The $IC_{50}$ values of water and methanol extracts were 0.79 and 0.36 mg/mL on HepG2 cells, 0.57 and 0.27 mg/mL on HT-29 cells, and 0.89 and 0.51mg/mL, respectively (Table 3). These $IC_{50}$ value results demonstrate the superiority of methanol extract to water extract in cell inhibitory activity against all tested cell lines. These findings also show that the most sensitive cell line for the plant extracts was HT-29 cells, followed by HepG2 and MCF-7 cells. On the other hand, both extracts were observed to show moderate cytotoxic activity compared to positive control 5-FU against three cell lines.

These in vitro results are in accordance with the data reported in the literature. Naghibi et al. (2014) reported a study on cytotoxic activities of methanolic extracts of three Gypsophila species against HepG2, HT-29, MCF-7 as well as A549 (lung cancer cell line) and MDBK (median- darby bovine kidney), and none of these extracts showed cytotoxic activity on the tested cell lines up to concentration of 0.1 mg/mL, while only Gypsophila bicolor had cytotoxicity on MDKB cells (Naghibi et al., 2014). In another study, methanol extract of Gypsophila capillaris was reported to inhibit the cell proliferations of MCF-7, HCT-116, HepG2 and A-549 cells by 32.5%, 10.3%, 24% and 10.8%, respectively at the concentration of 0.1 mg/mL (Moustafa et al., 2014). Furthermore, methanolic extract of Gypsophila trichotoma was reported to induce the inhibition of BV-173 leukemia cell proliferation by 2.2% at the concentration of 0.1 mg/mL (Voutquenne-Nazabadioko et al., 2013). Against the moderate cytotoxicity of the family Caryophyllaceae extracts, the saponins they possess was reported to enhance the cytotoxic potentials of the extracts (Ayaz et al., 2016; Holmes et al., 2015; Kamal et al., 2015). For instance, studies on saponins of Gypsophila species have illustrated their anti-carcinogenic properties, including

cytotoxicity, immune-modulating effects and normalization of carcinogen induced cell proliferation (Navarro del Hierro et al., 2018). The saponins in the extracts exploit their cytotoxic effects through either apoptosis inducement or non-apoptotic cell death stimulation. In fact, there are some well-known process leading to cell death, but having different mechanisms of action such as stimulation of autophagic cell death, decrease in nitric oxide (NO) production and disassembly of cytoskeleton integrity (Podolak et al., 2010). In conclusion, as far as our best knowledge, this is the first study to refer to the in vitro cytotoxic activity of Gypsophila aucheri extracts against the three tested cancer cell lines. The individual phytochemicals especially saponins as well as detected phenolics in the extracts that contribute to their biological activity, including antioxidant and anti-proliferative activity should be further analyzed in details by using biological activity guided chromatographic techniques.

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