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Investigation of the Biological Activities of *Alcea calvertii*

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ABSTRACT

Herbs are widely used in the treatment of diseases as colds, infections, and cancer. In this work, we evaluate *Alcea calvertii,* which is a perennial herbaceous plant belonging to the Malvaceae family. It spreads in Anatolia and Mediterranean region and has important uses in terms of ethnobotany. In this study, it was aimed to evaluate the cytotoxic potentials and to investigate the antioxidant activities of methanol, water, chloroform, and ethyl acetate extracts of the aerial parts of *Alcea calvertii*. For that, the antioxidant activity of *Alcea calvertii* was determined by four different methods [total phenolic content (TPC), ferric reducing antioxidant power (FRAP), copper reducing antioxidant capacity (CUPRAC) and 2,2-diphenylpicrylhydrazil (DPPH) radical scavenging activity. The cytotoxicity potential of extracts was assessed in the human lung cancer cell line (A549) by MTT assay. It was observed that the highest antioxidant activity was in the methanol extract and the antioxidant activity increased with increasing extract concentration; The TPC values were between 62.5 - 414.6 GAE µg mL^{-1}, the FRAP values were between 115.7 - 1321.4 µM Trolox equivalent g^{-1}, CUPRAC values were between 177.1 - 1321.4 µM Trolox equivalent g^{-1}, and IC_{50} values in DPPH determination were between 0.0089 - 3.5370 mg mL^{-1}. The extracts caused cytotoxicity in a concentration dependent manner, the IC_{50} values were calculated to be between 36.8 - 62.64 µg mL^{-1}. It is concluded that *Alcea calvertii* could be an important herb in developing new drugs.

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Alcea calvertii'nin Biyolojik Aktivitelerinin İncelenmesi

ÖZET

Bitkiler ve bitkisel ilaçlar soğuk algınlığı, enfeksiyonlar ve kanser gibi farklı hastalıkların tedavisinde kullanılmaktadır. *Alcea calvertii*, Malvaceae familyasına ait çok yıllık otsu bir bitkidir. Anadolu ve Akdeniz bölgesinde yayılan bu bitki etnobotanik açısından önemli bir yere sahiptir. Bu çalışmada, *Alcea calvertii* bitkisinin topraküstü kısımlarının metanol, su, kloroform ve etil asetat ekstrelerinin antioksidan aktiviteleri ile bu ekstrelerin sitotoksik potansiyellerinin incelenmesi amaçlanmıştır. Bu kapsamda, *Alcea calvertii*'nin antioksidan aktivitesi dört farklı yöntem [toplam fenolik içeriği (TPC), ferrik indirgeyici antioksidan gücü (FRAP), bakır indirgeyici antioksidan kapasitesi (CUPRAC) ve 2,2-difenilpikrilhidrazil (DPPH) radikal süpürme aktivitesini] ile tespit edilmiştir. İnsan akciğer kanseri hücre hattındaki (A549) sitotoksik potansiyeli ise MTT testi ile belirlenmiştir. En yüksek antioksidan aktivitenin metanol ekstresinde olduğu ve artan ekstre konsantrasyonu ile antioksidan aktivitenin arttığı gözlenmiştir; TPC değerlerinin 62,5- 414,6 GAE µg $mL^{-1,}$ FRAP değerlerinin 115,7-

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1321,4 µM Trolox eşdeğeri g^{-1}, CUPRAC değerlerinin 177,1 - 1321,4 µM Trolox eşdeğeri g^{-1}, DPPH tespitinde IC_{50} değerlerinin 0,0089-3,5370 mg mL^{-1} arasında hesaplanmıştır. Ekstreler konsantrasyona bağlı olarak hücre ölümüne neden olmuştur ve IC_{50} değerleri 36,8-62,64 µg mL^{-1} arasında bulunmuştur. Bu sonuçlar umut verici olup daha kapsamlı çalışmalar ile incelenmesinin önemli olduğu düşünülmektedir.

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INTRODUCTION

During the history of mankind, the interest in herbal therapies has been continued in progress, where the increases and decreases in the uses were following each other. Recently, an increase in herbal use among the different groups of society was observed. According to WHO reports about 70-80% of people prefers herbs primarily for health care. (Maiti et al., 2011). Different factors affect the increase in herbs production and usage, as the belief of low toxicity and the absence of the side effects of natural products (Verschaeve et al., 2004).

Alcea calvertii is a flowering herb of the Malveceae family and assumed to be one of the important plants in terms of ethnobotany in Anatolia. In Turkey, it is commonly known as "Gülhatmi" and distributed in Artvin, Elazig, Erzincan, Erzurum, Tunceli provinces (Akan et al., 2015; Konczak et al., 2014; Korkmaz et al., 2014; TÜBİVES). Although, *Alcea calvertii* thought to be Anatolia-endemic herb (Beğen and Yüksel, 2018; Uzunhisarcıklı and Vural, 2012) the studies in the other countries disproved that (Korkmaz et al., 2014; 2014b).

It was reported that some species of genus Althea or Alcea (Malveceae) have different pharmacological activities. *Althaea rosea,* one of the most studied Alcea species, was reported to have cytotoxicity (Abdel-Salam et al., 2018; Al-Snafi, 2013), anticancer (Shahbipour et al., 2017), antimicrobial (Al-Snafi, 2013; Mert et al., 2010), anti-influenza (Sargin, 2021), antioxidant (Abdel-Salam et al., 2018; Dar et al., 2017; Kordalı et al., 2020; Lee et al., 2018), anti-hyperglycemia (Dar et al., 2017), hepatoprotective effects against paracetamol induced toxicity (Hussain et al., 2014), and curative / protective activity in rats with urolithiasis by decreasing the calcium oxalate deposits; which thought to be in relation with the anti-inflammatory and diuretic effects of mucilaginous and polysaccharides in the herb (Ahmadi et al., 2012). Antitussive, antimicrobial, and anti-inflammatory effects were mentioned for Althaea officinalis (Al-Snafi, 2013). *Alcea pallida* was reported to have some antimicrobial activity (Ertas et al., 2016), anti-influenza (Sargin, 2021), expectorant and anti-inflammatory activity (Unal et al., 2008). Data show that *Alcea apterocarpa* acetone extract has a strong radical scavenging (Ertas et al., 2016), and similar activity was reported for *Alcea hyrcana* Grossh (Zakizadeh et al., 2011). Other studies reported anti hyperlipidemia and anti-hypercholesterolemia of *Alcea angulata* root ethanolic extracts (Fahimi et al., 2012; Fahimi et al., 2018). The *in vivo* studies indicated that *Alcea Aucheri* show an anxiolytic-like and sedative effects (Mombeini et al., 2017) anticonvulsant effects (Mombeini et al., 2020).

On the other hand, the ethnobotanical studies show that different parts of *Alcea calvertii are* used for its anti-inflammatory effect especially for cold, and for skin disorders, kidney stones, urinary system, pulmonary and stomach disorders. *Alcea calvertii* has been used mainly by the total herb, the aerial parts or the roots decoction (Ahmed et al., 2016; Altundag and Ozturk, 2011; Azab, 2016; Sargin, 2021), by the infusion of aerial parts, the total herb, the flowers and leaves (Altundag and Ozturk, 2011; Azab, 2016; Dalar et al., 2018; Sargin, 2021) or directly as a powder (Dalar et al., 2018). Additionally, it was shown that *Alcea calvertii* and other 9 of the Alcea genus in the Malvaceae family were frequently used because of their anti-urolithatic activity (Azab, 2016; Bozyel and Mert, 2018; Doğan and Tozlacı, 2015; Mossaddegh et al., 2012).

Previous studies prove that herbs with anti-urolithatic effects like *Bergenia ciliata* (Saxifragaceae) (Byahatti et al., 2013), *Pinus elderica* (Pinaceae) (Hosseinzadeh et al., 2010) and *Aerva lanata* (Amaranthaceae) (Saravanasingh et al., 2016) also have antioxidant, anti-bacterial and anti-inflammatory effects (Ahmed et al., 2016; Altundag and Ozturk, 2011; Konczak et al., 2014). Based on these data on the plant we hypothesized that *Alcea calvertii* might have an antioxidant effect. To test this hypothesis; the antioxidant activities of extracts have been determined by total phenolic content (TPC), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), and 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity. Besides that, the cytotoxic effect of *Alcea*

calvertii extracts was evaluated using MTT assays in the human lung cancer cell line (A549).

MATERIAL and METHOD

Material

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), dimethyl sulfoxide, acetic acid, acetonitrile, methanol, Trolox (6 hydroxy–2,5,7,8-tetramethylchroman–2-carboxylic acid), TPTZ (2,4,6-tripyridyl-s-triazine), trypan blue, and Folin-Ciocalteu's phenol reagent were purchased from Sigma Aldrich (St. Louis, MO, USA). Fetal bovine serum, Trypsin/ EDTA solution, antibiotic solution, and cell culture medium were purchased from Multicell Wisent (Quebec, Canada).

Herbal Extraction

Alcea calvertii were collected in July 2017 from the Erzincan (Turkey) and identified by Professor Ali Kandemir. The voucher specimens were kept in the herbarium of Erzincan University, Faculty of Science (Herbarium number: 10955). The aboveground parts of *Alcea calvertii* were dried in the shade, powdered in the grinder. 250 g of powder was extracted with 1000 mL methanol, water, chloroform, and ethyl acetate with continuous stirring for 24 hours at room temperature. The extracts filtered using Whatman No. 1 filter paper. The water phase and the methanolic phase were concentrated at 40°C under reduced pressure using a rotary evaporator. Plant extracts from the evaporator were used to prepare solutions at a concentration of 1, 5 and 10 mg mL^{-1} (Güner et al., 2019).

Antioxidant Activity

DPPH• Radical Scavenging Activity: DPPH radical-scavenging activity evaluation is based on the antioxidant's DPPH cation radical scavenging capacity (Molyneux, 2004). Briefly, 0.75 mL of DPPH reagent (0.1 mM in methanol) was added to 0.75 mL of all samples and standard, vortexed vigorously, and incubated for 40 mins at room temperature in the darkness. The discoloration of DPPH was measured spectrophotometrically at 517 nm. All measurements were carried out in triplicate. The DPPH• scavenging percentage was calculated using the formula (1):

DPPH• scavenging percentage=((A0 – A1)/A0)x 100(1)

where, A0 is the absorbance of the control, and A1 is the absorbance of the sample extracts. The exponential regression equation obtained after plotting the DPPH scavenging percentage as a function of concentration (mg mL^{-1}) was used to calculate the IC_{50}, indicating the concentration of samples giving rise to 50% scavenging of DPPH radicals.

Total Phenolic Content (TPC): The total phenolics amount in extracts was determined using the Folin-Ciocalteu procedure (Maiti et al., 2011; Singleton and Rossi, 1965). Phenolic compounds with Folin-Ciocalteu reagent form a blue color complex in an alkaline environment. This blue color can then be measured using the spectrophotometer, giving the total phenolic content. Gallic acid was used as a standard, and the total phenolics were expressed as µg of gallic acid equivalents (GAE) per g of sample. For that, 0.05 mL of each sample were placed into test tubes and mixed with 0.25 mL of 0.2 N Folin-Ciocalteu reagent and 0.75 mL of 7.5% sodium carbonate. Tubes were incubated for 2 h at room temperature, then the absorbance was read at 765 nm spectrophotometrically. The test was carried out in triplicate.

Ferric Ion Reducing Antioxidant Power (FRAP): Ferric ion reducing antioxidant power (FRAP) assay is based on the measurement of the iron-reducing capacities of the extract (Benzie and Strain, 1996; Korkmaz et al., 2014). FRAP reagent was obtained by combining 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Next, 0.05 mL of the samples was mixed with 1.5 mL of the freshly produced FRAP reagent and incubated at room temperature for 20 min. Absorbance was measured at 595 nm. The assay was carried out in triplicate. Trolox (in ethanol, at concentration 62.5 – 1000 µM) was used as standard and treated similarly. FRAP values were expressed as µM Trolox equivalent of g sample.

Cupric Reducing Antioxidant Capacity (CUPRAC): Reducing Mass levels of Antioxidant Capacity (CUPRAC) of the extracts was investigated using the method described previously (Apak et al., 2006; Doğan and Tuzlaci, 2015). Briefly, 1 mL of $CuCl_2$ solution (1.0 x10-2 M), 1 mL of neocuproine solution (7.5×10^{-3} M) and 1 mL of ammonium acetate buffer solution were added to a test tube and mixed. 0.5 mL samples or standard solutions were added and incubated for 30 minutes at room temperature. The absorbance was measured at 450 nm. The test was done in triplicate. The CUPRAC values were expressed as µM Trolox equivalent per gram of sample.

Cytotoxic Activity (MTT Test)

Human lung cancer A549 cell line was obtained from the American Type Culture Collection (CRL-1571TM, ATCC, USA). The cells were cultured in DMEM/F12 medium (Hyclone) containing 1% penicillin and 10% fetal bovine serum (FBS) at 37°C in 5% CO_2 and 95% O_2 humidified cell incubator. When the growth of cell monolayer reached 70%-80% confluence, 0.25% trypsin was used for digestion and passage.

The cytotoxicity of the extracts was evaluated by MTT assay which investigates the mitochondrial activity in the cells. The assay principle is that, in the presence of an electron-coupling reagent, the yellow water-soluble tetrazolium salt MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl-tetrazolium bromide, is reduced to an insoluble purple formazan product by mitochondrial succinate dehydrogenase, which belongs to the mitochondrial respiratory chain and is only active in the viable cells (Abudayyak et al., 2015; Alley et al., 1988).

For that, the cells were seeded in 96-well plates at 10^4 cells per well and allowed to attach overnight. Cells were treated with different concentrations (3.125, 6.25, 12.5, 25, 50 and 100 mg mL^{-1}) of herb extracts in DMSO. At the end of the exposure period (24 hours), 25 µL of MTT solution (5 mg mL^{-1}) was added to each well and incubated for a further 2 hours. After that, the supernatant was carefully aspirated, and the formazan crystals were dissolved in 100 µL DMSO. The optical density (OD) of the formazan product was read at 590 nm against the reference wavelength of 670 nm using a microplate reader. In every test, negative (untreated, culture medium) and solvent (1% DMSO) controls were used. Independent experiments were done in triplicate and repeated 3 times (n=9).

The inhibition concentration (IC) value was calculated as the percentage of solvent controls according to the formula (2), results were expressed as 50% inhibition concentration (IC_{50}), the concentration of samples that caused a 50% inhibition of enzyme activity in the cells.

Inhibition (%) = 100 – [(corrected mean OD sample × 100)/corrected mean OD solvent control] …… (2)

Statistical Analysis

Statistical analyses were carried out by one-way ANOVA Post Hoc Dunnett's test using SPSS v.20 for Windows (SPSS Inc., Chicago, IL). Data was expressed as mean ± standard deviation (SD). A two-tailed $p<0.05$ was considered to indicate a statistically significant difference (Abudayyak et al., 2015; Alpertunga et al., 2014).

RESULTS

Antioxidant Activity

Several mechanisms are often used to explain the antioxidant potential of a substance or a complex mixture; The main mechanisms involve the free radical scavenging, reduction capacity, and metals chelation as DPPH, CUPRAC, FRAP and TPC tests were used in this study to evaluate the antioxidant potential of herb extracts.

DPPH• Radical Scavenging Activity: DPPH radical scavenging activity was found to be concentration-dependent (Table 1). All the extracts were found to have radical scavenging activity of DPPH, the IC_{50} values of the extracts ranged from 0.0089 to 3.5370 mg mL^{-1}. Results show that MeOH extract of *Alcea calvertii* at a concentration of 10 mg mL^{-1} with the lowest IC_{50} value (0.0089 ± 0.0003 mg mL^{-1}) exhibits the highest antiradical activity against the DPPH free radical, while the $CHCl_3$ extract at a concentration of 1 mg mL^{-1}has the lowest activity with an IC_{50} value of 3.5370 ± 0.0145 mg mL^{-1}. The IC_{50} value of BHT used as the standard compound was 0.0325 mg mL^{-1}. In conclusion, the antioxidant effect of *Alcea calvertii* is 36.5 times greater than that of synthetic antioxidant BHT (Table 1).

Total Phenolic Content (TPC): The different extracts of *Alcea calvertii* gave TPC values with a total antioxidant capacity ranging between 62.50 and 414.58 GAE µg mL^{-1}. The antioxidant capacity was increased as the concentration of samples increased and samples with a concentration of 10 mg mL^{-1} have the highest antioxidant capacity. Besides that, MeOH extract showed higher antioxidant capacity than the other extracts, while EtAc extracts had the lowest (Table 1).

Ferric Ion Reducing Antioxidant Power: Alcea calvertii extracts gave FRAP values with a total antioxidant capacity ranging between 115.71 and 1321.43 µM Trolox equivalent g^{-1}. the antioxidant capacity was increased in a concentration-dependent manner. Also, MeOH extract showed higher antioxidant capacity than the other extracts and the $CHCl_3$ extract had the lowest (Table 1).

Cupric Reducing Antioxidant Capacity: The extracts of *Alcea calvertii* gave CUPRAC values with a total antioxidant capacity ranging between 177.14 and 2102.38 µM Trolox equivalent g^{-1}. The antioxidant capacity was increased as the concentration of samples increased, samples with a concentration of 10 mg mL^{-1} have the highest antioxidant capacity. Besides that, MeOH extracts showed the highest antioxidant capacity and the $CHCl_3$ extract had the lowest (Table 1).

Cytotoxic Activity

In the current study, different extracts of *Alcea calvertii* were evaluated for their cytotoxic potentials on A549 cell lineusing MTT assay. The tested concentrations were established based on the maximum permissible concentrations of the test conditions; and the following concentrations were used 3.125, 6.25, 12.50, 25.00 50.00 and 100.00 µg mL^{-1}.

The IC_{50} values were calculated to 36.82; 47.5; 62.64 and 50.47 µg mL^{-1} for MeOH, water, EtAc, and $CHCl_3$ extracts, respectively. Results showed that MeOH extract had the highest cytotoxic activity while EtAc extract showed the lowest (Figure 1).

Control cells were exposed to 1% DMSO. The statistical evaluation was performed by one-way ANOVA Post Hoc Dunnett's test, compared to the control group. The results were expressed as the means. Error bars represent ±standard deviation, (* $p< 0.05$).

Table 1: The antioxidant activities of *Alcea calvertii* extracts
Çizelge 1: *Alcea calvertii* ekstrelerinin antioksidan aktiviteleri

extracts	***ekstreler***	*mg mL^{-1}*	***TPC*** *GAE µg g^{-1}*	***FRAP*** *(TE µM g^{-1})*	***CUPRAC*** *(TE µM g^{-1})*	***DPPH*** *(mg mL^{-1})*
Methanol	*Metanol*	1	82.92 ± 1.47	510.00 ± 3.35	355.24 ± 2.37	0.2311 ± 0.0025
		5	192.5 ± 2.25	719.52 ± 5.11	847.14 ± 3.46	0.1022 ± 0.0021
		10	414.58 ± 2.7	1321.43 ± 6.4	2102.38 ± 7.6	0.0089 ± 0.0003
Water	*Su*	1	64.17 ± 0.92	359.05 ± 1.52	260.48 ± 1.58	0.3366 ± 0.0041
		5	127.5 ± 2.02	520.48 ± 3.48	603.33 ± 1.68	0.2635 ± 0.0027
		10	313.33 ± 2.4	964.76 ± 5.27	1029.05 ± 5.4	0.0563 ± 0.0012
ethyl acetate extract	*Etil asetat*	1	62.50 ± 0.73	160.48 ± 1.64	289.52 ± 1.96	2.6722 ± 0.0135
		5	170.83 ± 1.2	315.71 ± 2.31	361.43 ± 2.64	1.8282 ± 0.0127
		10	274.17 ±1.3	454.29 ± 3.14	657.14 ± 3.63	1.5779 ± 0.0115
chloroform	*Kloroform*	1	78.33 ± 0.67	115.71 ± 1.20	177.14 ± 1.82	3.5370 ± 0.0145
		5	127.1 ± 1.39	167.14 ± 1.63	204.29 ± 2.72	1.9159 ± 0.0093
		10	228.3 ± 1.47	504.29 ± 3.24	310.00 ± 1.39	1.3909 ± 0.0078
BHT	*BHT*					0.0325 ± 0.009

results were expressed as the means ±standard deviation .

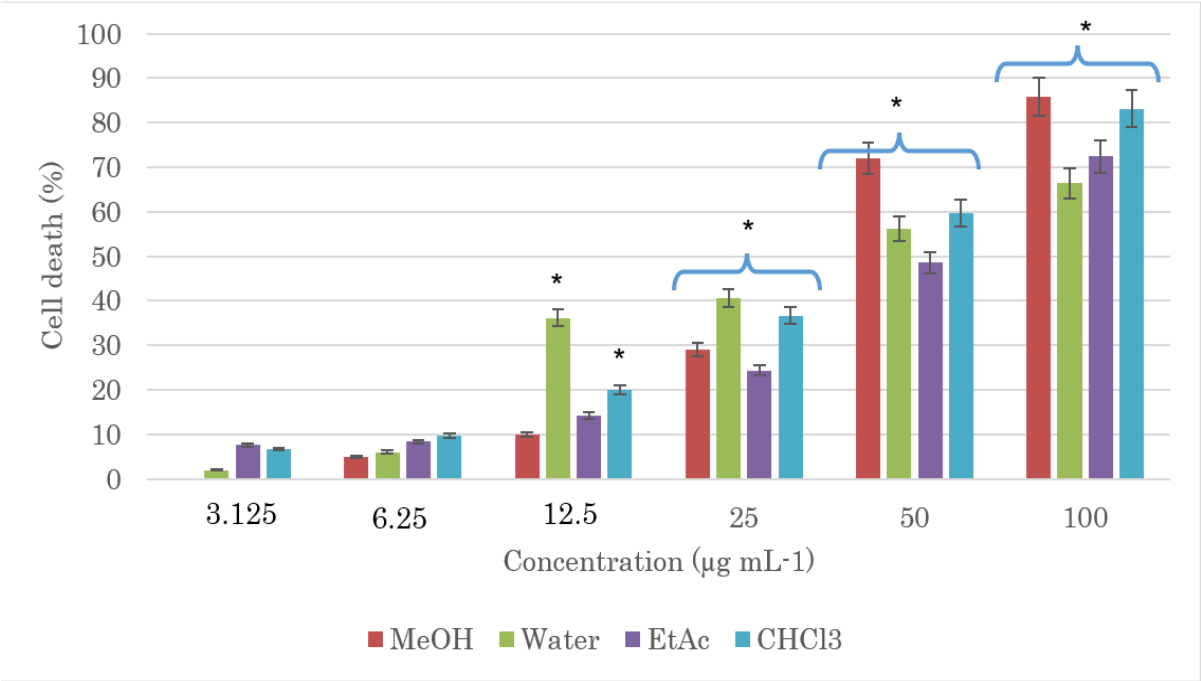

Figure 1: The cytotoxic activity of *Alcea calvertii* by MTT test in A549 cell line.
Şekil 1: A549 hücre hattında MTT testi ile *Alcea calvertii* 'in sitotoksik aktivitesinin değerlendirmesi.

DISCUSSION

About 70-80% of the world's population benefits from treatment with herbal resources. Especially in folkloric as Chinese medicine, herbs have been using as a medicine for the treatment of many diseases including cancer (Lau et al., 2004; Pan et al., 2010). Furthermore, herbal medicinal products have been using as remedies in both Eastern and Western cultures for hundreds of years (Chan, 2003).

People are exposed to the harmful effects of reactive oxygen species (ROS) by many sources. While ROS are produced by organisms that survive as a result of normal cellular metabolism (Halliwell and Gutteridge, 1999), the endogenous defense system of the human body shows weak effects against ROSs, depending on both aging and environmental factors. As a result, many of the components in the body are damaged and all of this is the cause of degenerative diseases.

Since ROS harms the organism depends on the

amount; When their amount is low or medium level, they do not continue to function physiologically and harm the organism. However, when ROS levels increase, they can create harmful effects on cell components such as DNA, protein, and lipid (Marnett, 1999; Valko et al., 2006). Oxidative stress caused by the shift of oxidant / antioxidant balance towards oxidants; causing cancer, neurological disorders (Lyras et al., 1997; Sayre et al., 2001), atherosclerosis, hypertension, ischemia/perfusion (Dhalla et al., 2000; Kasparova et al., 2005), diabetes and various lung diseases as pulmonary disorders, chronic obstructive lung disease (Asami et al., 1997). However, besides the protective antioxidant defense systems in the organism that neutralize these free radicals (Valioglu et al., 1998), components such as phenolic compounds and flavonoids found in plants are natural antioxidant agents play an impartment role in the defense against the free radicals. These components are known to have antimicrobial, anti-inflammatory, antiallergic, anti-inflammatory, anticarcinogenic, cytotoxic activities in addition to their antioxidant activities (Dillard and German, 2000; Prior, 2003; Rao et al., 2007). Phenolic compounds have been suggested to contribute by regulating carcinogen metabolism, inhibiting DNA binding, inducing apoptosis, and inhibiting the survival of cancer cells (Huang and Chai, 2010; Demir et al., 2017). For this reason, the demand for exogenous antioxidants is gradually increasing for the body to cope with oxidative stress (Zaporozhets et al., 2004). Epidemiological studies indicated that plant-weighted nutrition affects positively the human health and that this form of nutrition makes the human body more resistant to many diseases related to oxidative stress (Manach et al., 2005).

In this study, TPC, FRAP, CUPRAC, DPPH methods, which are widely used in determining antioxidant activity, were performed (Sener et al., 2018). Results show that the highest TPC, FRAP, CUPRAC and DPPH values of the *Alcea calvertii* methanolic extract (10 mg mL^{-1}) were found to be 414.58 ± 2.71 µg gallic acid equivalent g^{-1}, 1321.43 ± 6.39 µM Trolox equivalent g^{-1}, 2102.38 ± 7.56 µM Trolox equivalent g^{-1}, and 0.0089 ± 0.0003 mg mL^{-1}, respectively. The DPPH antioxidant effect of *Alcea calvertii* methanolic extract was found to be approximately 36 times greater than the effect of BHT, a synthetic antioxidant. Similar to this data, several studies indicate the antioxidant activity and free radical scavenging activity of different Alcea species. Studies have also reported that there is a powerful association between TPC and antioxidant activities (Giorgi et al., 2005; Scalzo et al., 2005). In a new study by Keser et al., (2020), the methanol, ethanol, and water extracts of *Alcea calvertii* flowers were evaluated for antiradical-antioxidant and antimicrobial and other activities. Their results indicate that the extracts at 500 µg mL^{-1} concentration show antioxidant activity but lower than Trolox the standard, and the extracts have a great antimicrobial activity against different microorganisms (Keser et al., 2020). In a study by Tusevski et al (2004), they found the total phenolic content of the flowers of *Alcea pallida* 10.90 ± 0.36 mg GAE g^{-1} dry weight (DW), CUPRAC value 52.89 ± 1.08 µmol TE g^{-1} DW, DPPH value 56.73 ± 0.68 µmol TE g^{-1} DW (Tusevski et al., 2004). Qader and Awad (2014) have demonstrated the aqueous extract of *Alcea kurdica* has potent free radical scavenging activity of 64% ± 1.64% and ferric reduction capacity of 2955.0 ± 0.04 mmol g^{-1}, as well as having 88.0 ± 0.002 mg gallic acid equivalents g-1 plant extract. They have reported the TPC value of *A. kurdica* was 80 ± 0.98 mg gallic acid equivalents g^{-1} of the extract (Qader and Awad, 2014). In similar to this study findings, Qader et al. reported that the potent radical scavenging effect is positively associated with the high content of phenolic components (Qader et al., 2011).

Alcea setosa, one of Alcea species that widely used in the Mediterranean folk-medicine for kidney stone, urinary tract disorders and pulmonary diseases, was evaluated by Alhage & Elbitar (2019) and weak antioxidant activity of was reported (Alhage and Elbitar, 2019). Azadeh et al., (2020) evaluate the radical scavenging activity of three Alcea species Using DPPH assay, and they found a high scavenging activity in *A. aucheri* var. aucheri (IC_{50} 34.06 µg mL^{-1}) (Azadeh et al., 2020). Anlas et al., (2017) reported that the total amount of phenol content and the DPPH radical scavenging activity of *Alcea apterocarpa* (FENZL) BOISS, one of the Turkey endemic herbs, varied according to extraction method, and TPC ranged from undetectable to 33.28 ± 2.55 mg gallic acid g^{-1} dry weight, while the IC_{50} of DPPH radical scavenging activity test was 379.7±2.00 - 1820±2.15 µg mL^{-1} (Anlas et al., 2017).

Abdel-Salam et al., (2018) isolated six flavonoids from the flowers of *Alcea rosea* L. and evaluated their antioxidant, Immune stimulant, and cytotoxicity potential in HepG-2 hepatocarcinoma cells and normal peripheral blood mononuclear cells (PBMC); Their results indicated a significant immune stimulant activity for some compounds and significant antioxidant activity for others. Additionally, the isolated flavonoids showed cytotoxicity against the cells with IC_{50} arranged between 3.82 to 374.75 µg mL^{-1} in HepG-2 cells and 58.46 to 193.49 in PBMC cells; They also reported a high selectivity towards the cancer cells for kaempferol-3-O-ß-d-glucopyranoside the flavonoid with the highest cytotoxicity (Abdel-Salam et al., 2018). Yaglioglu et al., (2016) evaluated its methanol extract against brain tumor C6 cells an human

cervical cancer HeLa cells and the IC_{50} values were 37.63 and 14.48 µg mL^{-1}, respectively. For the same herb, Kalemba-Drożdż & Cierniak (2019) found that 100-folds diluted 1% flower tincture did not induce any cytotoxicity in human peripheral blood cells after treatment for 1& 24 h (Kalemba-Drożdż and Cierniak, 2019; Yaglioglu et al., 2016). Mert et al., (2010) used the brine shrimp lethality test to evaluate the toxicity of *Alcea rosea* L. extracts. They found that LC_{50} was 545.4 µg mL^{-1} for ethyl acetate extract and higher than 1000 µg mL^{-1} for methanol, ethanol and hexane extracts (Mert et al., 2010).

The malvidin-3,5-diglucoside that isolated from *Alcea longipedicellata* show a concentration-dependent cytotoxicity after 24h exposure period in AGS-C131 cells and the IC_{50} value was 140.6 µM (Karamani et al., 2008).

Qader and Awad (2014) evaluated the aqueous extract of *Alcea kurdica* Alef for the cytotoxicity potential in human normal fibroblast Hs888Lu cell line by Promega Cell Titer 96 AQueous Non-Radioactive Cell Proliferation MTS assay and reported no cytotoxicity at concentrations up to 2000 µg mL^{-1} (Qader and Awad, 2014). Similarly, *Alcea setosa* dichloromethane and methanol (1:1, v/v) extract was reported that to have "no toxicity" in L929sA, MCF7 and MDA-MB 231 cells lines after 24 h (Kaileh et al., 2007),

Esmaeili et al., (2016) evaluated the cytotoxicity of *Alcea calvertii* (Boiss.) methanolic extract against HepG-2, MCF-7, HT-29 and A549 cell lines at concentrations between 3.125 to 100 µg mL^{-1}; However, the IC_{50} values could not be calculated at the tested concentration (Esmaeili et al., 2016). In the present work, the IC_{50} value of methanolic extract calculated to be 36.82 µg mL^{-1}. This difference could be explained by the differences in the season of collection, the collection area, or the used part.

Keser et al., (2020) evaluated the antiproliferative activity of *Alcea calvertii* flowers methanol, ethanol, and water extracts in MCF-7, HCT-116 and LNCaP cell lines. The IC_{50} values of extracts calculated to be 15.25 – 25.17 µg mL^{-1} MCF-7, 9.89 – 11.84 µg mL^{-1} in LNCaP and 10.78 – 20.08 µg mL^{-1} in HCT-116 cells, where methanol extracts the potent in LNCaP cells and water extract in the other cell lines (Keser et al., 2020). Similarly, in the present study the methanol extract is the most potent in A549 cells, and IC_{50} values calculated to be 36.82; 47.5; 62.64 and 50.47 µg mL^{-1} for MeOH, water, EtAc, and $CHCl_3$ extracts, respectively; Which confirm with the antioxidant activity results, since methanol extract show the highest radical scavenging activity and contains the highest amount of TPC, and also exhibit the best activity by FRAP and CUPRAC assay. Results show also close antioxidant activity of ethyl acetate and chloroform extracts which was lower than other extracts activity and parallel with cytotoxicity activity.

CONCLUSION

Nowadays, the usage of herbal products was increase dramatically. Besides the traditional uses, herbs have been used in the development of modern drugs. *Alcea calvertii* is one of commonly used herbs in Turkey and Middle East countries for treat different diseases like anti-urolytic. From the previous data it was noticed that herbs with anti-urolytic activity also show antioxidant activities; For that, in this work it was hypothesized that *Alcea calvertii* has an antioxidant activity. Additionally, as a preliminary study of antineoplastic activity, the cytotoxic potential of *Alcea calvertii* extracts was estimated by MTT assay in human lung cancer cells. This study is the first work that evaluates the antioxidant potential and cytotoxic of *Alcea calvertii* in human lung carcinoma cells. The present results showed that the different extracts of *Alcea calvertii* have a cytotoxic activity against A549 cells and as we hypothesized, similar to the other anti-urolytic herbs as Bergenia ciliate, Pinus elderica and Aerva lanata, *Alcea calvertii*, has good antioxidant activity. The antioxidant activity values that obtained from this study are compatible with the literature data. It is thought that the characteristics of the soil on which the plant is grown, the climatic characteristics of the region where the plant is collected, the height differences on which the plant grows, and the type of solvent used are different. Therefore, the antioxidant capacity of *Alcea calvertii* could be related to its phenolic content. *Alcea calvertii* could be researched in order to develop new drugs. For that, more pharmacognostic, *in vivo* and *in vitro* studies are required to obtain active compounds of the herb and to investigate their pharmacological effects and to evaluate its safety.

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All authors contributed substantially to the manuscript and have met the criteria for authorship.

Statement of Conflict of Interest:

The authors declare that there are any competing interests.

REFERENCES

Abd El-Salam NM, Radwan MM, Wanas A, Shenouda M, Sallam S, Piacente S, ElSohly M, Ghazy N 2016. Phytochemical And Biological Evaluation of

Alcea rosea L., Growing In Egypt. Planta Medica 82:2–3.

Abdel-salam NA, Ghazy NM, Sallam SM, Radwan MM, Wanas AS, ElSohly MA, El-Demellawy MA, Abdel-Rahman NM, Piacente S, Shenouda ML 2018. Flavonoids Of Alcea rosea L. And Their Immune Stimulant, Antioxidant and Cytotoxic Activities On Hepatocellular Carcinoma Hepg-2 Cell Line. Natural Product Research 32:702–706.

Abudayyak M, Ozdemir Nath E, Ozhan G 2015. Toxic Potentials of Ten Herbs Commonly Used Aphrodisiac Effect in Turkey. Turkish Journal of Medical Sciences 44: 496-506.

Ahmadi M, Rad AK, Rajaei Z, Hadjzadeh MAR, Mohammadian N, Tabasi NS 2012. Alcea rosea Root Extract As A Preventive And Curative Agent In Ethylene Glycol-Induced Urolithiasis In Rats. Indian Journal of Pharmacology 44:304–307.

Ahmed S, Hasan MM, Mahmood ZA 2016. Antiurolithiatic Plants in Different Countries and Cultures. Journal of Pharmacognosy and Phytochemistry 5: 102-115.

Akan H, Bakır Sade Y 2015. Ethnobotanical Investigation of Kahta (Adıyaman) Center and Narince Village [Kahta (Adıyaman) Merkezi ve Narince Köyü'nün Etnobotanik Açıdan Araştırılması] Bitlis Eren University Journal of Science 4(2): 219-248.

Alpertunga B, Kara M, Abudayyak M, Oztas E, Ozden S, Özhan G 2014. Effects of prochloraz on DNA damage, lipid peroxidation and antioxidant system in vitro, Toxicology Mechanisms and Methods, 24:4, 268-275

Alhage J, Elbitar H 2018. In Vitro Screening for Antioxidant and Antimicrobial Properties of Three Lebanese Medicinal Plants Crude Extracts 2019. Pharmacognosy Research 11(2):127–133.

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR 1988. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Research 48: 589–601.

Al-Snafi AE 2013. The Pharmaceutical importance of Althaea officinalis and Althaea rosea: A review. International Journal of PharmTech Research 5:1378–1385

Altundag E, Ozturk M 2011. Ethnomedicinal Studies on The Plant Resources of East Anatolia, Turkey. Procedia - Social and Behavioral Sciences 19: 756-777.

Anlas C, Ustuner O, Alkan F, Bakirel T, Aydogan M, Erel S 2017. a Comparative Study on the Antioxidant Activities and Phenolic Contents of Different Extracts of a Chillea nobilis Subsp Sipylea and Alcea apterocarpa (Fenzl) Boiss, Endemic Plants in Turkey. Fresenius Environmental Bulletin 26:1423–1430.

Apak R, Güçlü K, Ozyürek M, Karademir SE, Erça E 2006. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. international journal food science nutrition 57:292-304.

Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, Yamaguchi R, et al 1997. Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. Carcinogenesis 18:1763–1766.

Azab A 2016. Alcea: traditional medicine, current research and future opportunities. European Chemical Bulletin 6 (5): 505-514.

Azadeh Z, Saeidi K, Lorigooini Z, Kiani M, Maggi F 2020. Organ-Oriented Phytochemical Profiling and Radical Scavenging Activity of Alcea spp. (Malvaceae) from Iran. SN Applied Sciences 2:1–9. https://doi.org/10.1007/s42452-020-2410-3

Beğen HA and Yüksel E 2018. The flora of Alanbaşı and Bakırtepe villages (Yusufeli, Artvin, Turkey) and its surroundings. Turkish Journal of Biodiversity 1:17–23.

Benzie IFF, Strain JJ 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239: 70-6.

Boyzal ME and Mert EM 2018. Antiurolithiatic Activity of Medicinal Plants in Turkey. (Assessment of Working Postures of Nursery Workers in Seedling Production Activities (Ed. Christov I, Strauss E, Gad A, Curebal I) 152-162.

Byahatti VV, Vasantakumar Pai K, D'Souza MG 2010. Effect of Phenolic Compound from Bergenia cliata (Haw.) Stemb. Leaves on Experimental Kidney Stones. Ancient science of life 30: 14-17.

Chan K 2003. Some aspects of toxic contaminants in herbal medicines, Chemosphere 52: 1361–1371.

Dalar A, Mukemre M, Unal M, Ozgokce F 2018. Traditional Medicinal Plants of Ağrı Province, Turkey. Journal of Ethnopharmacology 226:56–72.

Dar PA, Ali F, Sheikh IA, Ganie SA, Dar TA 2017. Amelioration of Hyperglycaemia and Modulation of Antioxidant Status By Alcea Rosea Seeds in Alloxan-Induced Diabetic Rats. Pharmaceutical Biology 55:1849–1855 . https://doi.org/10.1080/13880209.2017.1333127

Dhalla NS, Temsah RM, Netticadan T 2000. Role of oxidative stress in cardiovascular diseases. Journal of Hypertension 18: 655–673.

Doğan A, Tuzlacı E 2015. Some Local Plant Names of Tunceli [Tunceli'nin Bazı Yöresel Bitki Adları]. Eurasscience Journal 3: 22-33.

Ertas A, Boga M, Gazioglu I, Yesil Y, Hasimi N, Ozaslan C, Yilmaz H, Kaplan M 2016. Fatty acid, Essential Oil and Phenolic Compositions of Alcea Pallida and Alcea apterocarpa With Antioxidant, Anticholinesterase and Antimicrobial Activities. Chiang Mai Journal of Science 43:89–99 .

Esmaeili S, Irani M, Zehan HM, Keramatian B, Harandi ZT, Hamzeloo-Moghadam M 2016. Cytotoxic Activity of Some Ethnic Medicinal Plants From Southwest of Iran. Research Journal of Pharmacognosy 3:43–47

Fahimi Z, Cheraghi j, Pilehvarian AA, Sayehmiri K, Kjosarvi A 2012. Effects of Alcea angulate Roots Alcoholic Extract on Blood Lipid of Male Rabbit. Journal of Ilam University of medical Sciences 20:23-32.

Fahimi Z, Soltanbeigi S, Asadollahi K, Kaffashi M and Kaffashian MR 2018. Effects of Alcoholic Extract of Stem and Aerial Parts of the Alcea angulate Plant on Aorta Atherosclerotic Plaque Formation in Rabbit. Archives of Medicine 10:1- 5.

Giorgi M, Capocasa F, Scalzo J, Murri G, Battino M, Mezzetti B 2005. The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach. Scientia Horticulturae 107: 36-42.

Güner M, Kalaycioglu AT, Kanbolat S. orkmaz N, Aliyazicioglu R, Abudayyak M, et al 2019. Evaluation of Antioxidant, Antimicrobial, Antityrosinase and Cytotoxic Potentials of Isatis cappadocica subsp. alyssifoli as a Potent Pharmaceutical Resource. Journal of Pharmaceutical Research International 26: 1-12.

Halliwell B and Gutteridge JMC 1999. Free Radicals in Biology and Medicine. In: Halliwell B and Gutteridge JMC, Eds., Free Radicals in Biology and Medicine, 3rd Edition, Oxford University Press, Oxford, 1999:1-25.

Hosseinzadeh H, Khooei AR, Khashayarmanesh Z, Motamed-Shariaty V 2010. Antiurolithiatic Activity of Pinus elderica Medw. Fruits Aqueous Extract in Rats. Urology Journal 7: 232-7.

Hussain L, Akash MSH, Tahir M, Rehman K, Ahmed KZ 2014. Hepatoprotective Effects of Methanolic Extract of Alcea Rosea Against Acetaminophen-Induced Hepatotoxicity in Mice. Bangladesh Journal of Pharmacology 9:322–327 . https://doi.org/10.3329/bjp.v9i3.19068

Kaileh M, Berghe W Vanden, Boone E, Essawi T, Haegeman G 2007. Screening of Indigenous Palestinian Medicinal Plants for Potential Anti-Inflammatory and Cytotoxic Activity. Journal of Ethnopharmacology 113:510–516. https://doi.org/10.1016/j.jep.2007.07.008

Kalemba-Drożdż M, Cierniak A 2019. Antioxidant and Genoprotective Properties of Extracts From Edible Flowers. Journal of Food and Nutrition Research 58:42–50

Kamrani Y, Esmaeelian B, Jabbari M, Tabaraei B, Yazdanyar A, Ebrahimi S 2008. Anti-Cancer Effects of Malvidin-3,5-Diglucoside From Alcea Longipedicellata, on Gastric Cancer Cell Line (AGS). Planta Medica 74(9):A174. https://doi.org/10.1055/s-0028-1084172

Kasparova S, Brezova V, Valko M 2005. Study of the oxidative stress in a rat model of chronic brain hypoperfusion. Neurochemistry International 46: 601–611.

Keser S, Keser F, Tekin S, Kaygili Ö, Turkoglu I, Demir E, Karatepe M, Kirbag S, Yilmaz Ö, Sandal S, Turkoglu S 2020. In vitro Antiradical, Antimicrobial and Antiproliferative Activities and Phytochemical Compositions of Endemic *Alcea calvertii* (Boiss) Boiss. Flowers 2020. Düzce University Journal of Science & Technology Research 8:693–701. . https://doi.org/10.29130/dubited.595147

Konczak I, Dalar A, Konczak-Islam KA. "Health Attributes, Antioxidant Properties and Phytochemical Composition of Traditional Medicinal Plants from Eastern Anatolia", in Medicinal Plants, edt. Pereia D.A.M., Nova Science Publishers, 2014:183 – 225 p. ISBN: 978-1-62948-219-4

Kordali Ş, Usanmaz Bozhüyük A, Beyzi E, Güneş A, Turan M 2021. Antioxidant Enzyme, Phenolic Substance and Plant Nutrient Contents of Malva sylvestris L. and Alcea rosea L. Species Used as Medicinal Plants. Journal of the Institute of Science and Technology11:786–794 . https://doi.org/ 10.21597/jist.747673

Korkmaz M, Kandemir A, Karacan S 2014. A Surway on Determining The Plant of Taxa Zetrin Spice Ussed in Kemaliye District (Erzincan, Turkey). Bothalia Journal 44: No 3.

Korkmaz M, Karakurt E 2014b. Medicinal Plants Sold in Kelkit (Gümüşhane) [Kelkit (Gümüşhane) Aktarlarında Satılan Tıbbi Bitkiler]. Süleyman Demirel University Journal of Natural and Applied Sciences 18: 60-80.

Lau CBS, Ho CY, Kim CF, Leunge KN, Fung KP, Tse TF, Chow MSS 2004. Cytotoxic Activities of Coriolus versicolor (Yunzhi) Extract on Human Leukemia and Lymphoma Cells By Induction of Apoptosis. Life Science Journal 75: 797-808.

Lee G, Yeom A, Won K, Park CM, Joung MS, Lee GY, CP Jeong 2018. Evaluation of Alcea rosea L. Callus Extract as a Natural Cosmetic Ingredient. Journal of Society of cosmetic scientists of Korea 44:295–302

Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B 1997. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. Neurochemical Research 68: 2061–2069.

Maiti B, Nagori BP, Singh R, Kumar P 2011, Upadhyay N. Recent trends in herbal drugs: a review. The International Journal of Drug Research and Technology1: 17–25.

Manach C, Williamson G, Morand C, Scalbert A, Rémésy C 2005. Bioavailability and Bioefficacy of Polyphenols in Humans. I. Review of 97

Bioavailability Studies. The American Journal of Clinical Nutrition 81: 230-242.

Marnett LJ 1999. Lipid peroxidation DNA damage by malondialdehyde. Mutation Research 424: 83–95.

Mert T, Fafal T, Kivçaks B, Öztürk HT 2010. Antimicrobial and Cytotoxic Activities of The Extracts Obtained From The Flowers of Alcea rosea L. Hacettepe University Journal of the Faculty of Pharmacy 30:17–24

Molyneux P 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology 26: 211-9.

Mombeini T, Behzadi BA, Ejtemaei R, Tahmasbi F, Kamalinejad M, Dehpour AR 2020. Anticonvulsant Effect of Alcea aucheri on Pentylenetetrazole and Maximal Electroshock Seizures in Mice. Basic and Clinical Neuroscience11:369–378 .

Mombeini T, Pourbadie HG, Kamalinejad M, Mazloumi S, Dehpour AR 2017. Anxiolytic-Like and Sedative Effects of Alcea aucheri (Boiss.) Alef. Flower Extract in The Laboratory Rat. Iranian Journal of Pharmaceutical Research 16:1495–1508 . https://doi.org/10.22037/ijpr.2017.2142

Mossaddegh M, Naghibi F, Moazzeni H, Pirani A, Esmaeili S 2012. Ethnobotanical Surway of Herbal Remedies Traditionally Used in Kohghiluyehva Boyer Ahmad Province of Iran. Journal of Ethnopharmacology 141: 80-96.

Pan L, Chai H, Kinghom AD 2010. The Continuing Search for Antitumor Agents From Higher Plants. Phytochemistry Letters 3: 1-8.

Qader SW, Abdulla MA, Chua LS 2011. Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. Molecules 16: 3433-3443.

Qader SW, Awad HM 2014. Evaluation of Antioxidant, Antimicrobial and Cytotoxicity of Alcea kurdica Alef. Jordan Journal of Biological Sciences 7: 205-209.

Sahin Yaglıoglu A, Eser F, Tekin S, Onal A 2016. Antiproliferative Activities of Several Plant Extracts From Turkey on Rat Brain Tumor and Human Cervix Carcinoma Cell Lines. Frontiers in Life Science 9:69–74 . https://doi.org/10.1080/21553769.2015.1089949

Saravanasingh K, Ramamurthy M, Parthiban P 2016. In-vitro Anti-Urolithiatic Activity of Aerial Parts of Aerva lanata (L.) International Journal of Current Research in Medical Sciences 2: 24-27.

Sargin SA 2021. Potential Anti-Influenza Effective Plants Used ın Turkish Folk Medicine: A Review. Journal of Ethnopharmacology 265:113319. https://doi.org/10.1016/j.jep.2020.113319

Sayre LM, Smith MA, Perry G 2001. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Current Medicinal Chemistry 8: 721–738.

Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21: 207-213.

Sener SO, Korkmaz N, Akkaya S, Badem M, Aliyazicioğlu R, Özgen U, et al 2018. Investigation of Phenolic Compounds by RP-HPLC and Antioxidant, Antimicrobial, Tyrosinase Inhibitor Activities of Clinopodium vulgare L. subsp. vulgare Extract. [Clinopodium vulgare L. subsp. vulgare ekstresinin antioksidan, antimikrobial, tirozinaz inhibitor aktiviteleri ve rp-hplc ile fenolik bileşiklerinin araştırılması]. Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi 8: 230-238.

Shahabipour F, Caraglia M, Majeed M, Derosa G, Maffioli P, Sahebkar A 2017. Naturally Occurring Anti-Cancer Agents Targeting EZH2. Cancer Letters 400:325–335 . https://doi.org/10.1016/j.canlet.2017.03.020

Singleton VL, Rossi JA 1956. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-58.

Turkish Plants Data Service (TÜBİVES), Alcea calvertii. http://www.tubives.com/ , (accessed at March 02, 2020)

Tusevski O, Kostovska A, Iloska A 2004. Phenolic production and antioxidant properties of some Macedonian medicinal plants. Central European Journal of Biology 9: 888-900.

Unal EL, Mavi A, Kara AA, Cakir A, Şengül M, Yildirim A 2008. Antimicrobial and Antioxidant Activities of Some Plants Used As Remedies in Turkish Traditional Medicine. Pharmaceutical Biology 46:207–224 . https://doi.org/10.1080/13880200701735577

Uzunhisarcıklı ME, Vural M 2012. The Taxonomic Revision of Alcea and Althaea (Malvaceae) in Turkey. Turkish Journal of Botany 36: 603-639.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemistry & Biology 160: 1–40.

Verschaeve L, Kestens V, Taylor JLS 2004. Investigation of the antimutagenenic effects of selected South African medicinal plant extracts. Toxicology in vitro 18: 29-35.

Zakizadeh M, Nabavi SM, Ebrahimzadeh MA 2011. In vitro Antioxidant Activity of Flower Seed and Leaves of Alcea hyrcana Grossh. European Review for Medical and Pharmacological Sciences 15:406–412

Zaporozhets OA, Krushynska OA, Lıpkovska NA, Barvınchenko VN 2004. A New Test Method for the Evaluation of Total Antioxidant Activity of Herbal Products. Journal of Agricultural and Food Chemistry 52: 21−25 21.