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Research Article

In vitro Antiradical, Antimicrobial and Antiproliferative Activities and Phytochemical Compositions of Endemic *Alcea calvertii* (Boiss) Boiss. Flowers

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ABSTRACT

Alcea calvertii (Boiss) Boiss. is belonged to Malvaceae family, and it is a perennial herbaceous endemic plant. *Alcea* genus plants are grown as ornamental plants in the gardens. In the present study, the antiproliferative, antimicrobial, antiradical activities and phytochemical compositions of ethanol, water, methanol and acetone extracts of *A. calvertii* flowers were examined. *A. calvertii* flowers water, ethanol, methanol and acetone extracts are lower scavenged DPPH, ABTS and OH radicals than standard antioxidant trolox. *A. calvertii* flowers contain vitamins, sterols, flavonoids and phenolic acids, dominated by vanillic acid, gallic acid, catechin, δ-tocopherol, ergosterol and vitamin D. *A. calvertii* flowers water extract showed better antiproliferative activities than other extracts against to MCF-7 and HCT-116 cell lines. *A. calvertii* flowers methanol extract showed higher antiproliferative effect against to LNCaP cell lines.

Keywords: *Alcea calvertii, Antiradical, Antimicrobial, Antiproliferative, Phytosterol.*

Endemik *Alcea calvertii* (Boiss) Boiss. Çiçeklerinin *In vitro* Antiradikal, Antimikrobiyal ve Antiproliferatif Aktiviteleri ve Fitokimyasal Kompozisyonu

ÖZET

Alcea calvertii (Boiss) Boiss. Malvaceae familyasında yer alan çok yıllık otsu endemik bir bitkidir. *Alcea* genusuna ait bitkiler bahçelerde süs bitkileri olarak yetiştirilirler. Sunulan çalışmada, *Alcea calvertii* çiçeklerinin su, etanol, metanol ve aseton ekstraktlarının antiproliferatif, antimikrobiyal, antiradikal aktiviteleri ve fitokimyasal kompozisyonları incelendi. *A. calvertii* çiçeklerinin su, etanol, metanol ve aseton ekstraktlarının standart antioksidan trolokstan daha düşük oranda DPPH, ABTS ve OH radikali yok ettiği saptandı. *A. calvertii*

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çiçeklerinin vitamin, sterol, flavonoit ve fenolik asitler içerdiği ve yüksek miktarda vanillik asit, gallik asit, kateşin, δ-tokoferol, ergosterol ve vitamin D içerdiği belirlendi. *A. calvertii* su ekstraktının MCF-7 ve HCT-116 kanser hücrelerine karşı diğer ektstraktlardan daha iyi antiproliferatif aktivite gösterdiği gözlendi. LNCaP hücre serilerine karşı ise *A. calvertii* metanol ekstraktı daha yüksek antiproliferatif aktivite gösterdi.

Anahtar Kelimeler: *Alcea calvertii, Antiradikal, Antimikrobiyal, Antiproliferatif, Fitosterol.*

I. INTRODUCTION

Malvaceae is a family including about 200 genera and 2300 flowering plant species [1]. This family is represented 14 species in Turkey, and one of which is *Alcea* genus. There are twenty species of *Alcea* genus grown in Turkey, and 2 of them are endemic plants. *Alcea calvertii* is one of the endemic species [2,3]. The genus *Alcea* is distributed in the Mediterranean and Iran-Turan phytogeographic regions of the world. The species of this genus are also grown, in the Europe, North America, North Africa, Caucasus and South Russia [4].

The species belonging to the genus *Alcea* are grown as ornamental plants in the gardens. It is also known that these species are used in the treatment of colds, cough, stomach pain, inflammation and asthma among the people [5]. It has been shown that the plants of this genus have antiviral, antiinflammatory, diuretic and antimicrobial properties [6].

Nowadays, the investigation of medicinal properties of plants, and to benefit from therapeutic effects in the alternative medicine have been gained a great importance since the plants have numerous bioactive phytochemical compounds, which are used in the treatment of many diseases. The investigation of the antiradical, antiproliferative and antimicrobial properties and phytochemical compounds of the plants has great importance in the scientific world.

As far as we know, there is no report about the antiradical, antimicrobial and antiproliferative properties of *A. calvertii*. The goal of the present study is to investigate the antiproliferative, antimicrobial, antiradical activities, and phytochemical compositions of *A. calvertii* flowers ethanol, water, methanol and acetone extracts.

II. MATERIALS AND METHOD

A. STANDARDS AND CHEMICALS

All standards and chemical compounds were obtained from Sigma-Aldrich.

B. EXTRACTION PROCEDURES

Alcea calvertii flowers were collected in June 2016 from Elazig in Turkey. Voucher specimen is Turkoglu 4870, and they were stored in the herbarium of Firat Uni. Science Faculty, Biology Department, Elazig, Turkey. *A. calvertii* flowers were dried at dark and 25 °C. The parts were pulverized using a mechanic grinder and then 25 g of powdered samples were extracted with 250 mL of the solvent (water, ethanol, methanol and acetone) in the Soxhlet extractor (Gerhardt Soxtherm SOX-402, Germany). After filtrating of solvents, the supernatants were concentrated in a rotary evaporator to dry. The standard antioxidant and dried extracts were dissolved in DMSO (µg/mL).

C. DETERMINATION OF ANTIRADICAL ACTIVITIES

The antiradical activities (RSAs) were determined by the methods of Re et al. [7], Halliwell et al. [8] and Brand-Williams et al. [9] for ABTS, OH and DPPH radicals, respectively. All tests were repeated thrice and the average values were calculated.

The radical scavenging activity percentages (RSA%) for each sample were estimated using the following equation:
$RSA\% = [(A_0 - A_1)/A_0] \times 100$
A_0 and A_1 are the absorbance of control and the sample, respectively.

D. PHYTOCHEMICAL CONTENT ANALYSES

Total Phenolic Compounds (TPC): TPC in *A. calvertii* flowers were determined according to Slinkard and Singleton's method [10]. The TPC results are calculated as gallic acid equivalent.

Total Flavonoid Compounds (TFC): TFC were determined according to Kim et al.'s method [11]. The results are expressed as catechin equivalent.

Total Proanthocyanidin Compounds (TPAC): Determination of TPAC was carried out according to Amaeze et al.'s method [12]. The results are expressed as catechin equivalent.

Flavonoid, Non-Flavonoid and Phenolic Acids: The flavonoid, non-flavonoid and phenolic acids in the *A. calvertii* extracts were done using according to the method of Zu et al. [13] with HPLC. Morin, catechin, quercetin, resveratrol, gallic, hydroxycinnamic, vanillic, ferulic, rosmarinic and caffeic acid were determined by HPLC.

Fatty Acids Contents: Fatty acids contents in the *A. calvertii* flowers (%) were analyzed by GC according to Christie's method [14]. The fatty acid contents were expressed as percent.

Analyses of Phytosterols and Vitamins: Vitamins and phytosterols were extracted from the *A. calvertii* according to the HPLC method of Lopez-Cervantes et al. [15] and Sánchez-Machado et al. [16]. The results of the analyses were expressed as µg/g.

E. ANTIMICROBIAL ACTIVITY

B. megaterium, *E. coli*, *B. subtilis*, *P. vulgaris*, *L. monocytogenes*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* bacteria and *C. albicans* yeast were used as test organisms. Collins and Lyne's method [17] was used for the antimicrobial tests using the disc diffusion method. Nystatin (30 mg/disc) and streptomycin sulfate (10 mg/disc) were used as standard antibiotics.

F. ANTIPROLIFERATIVE PROPERTIES

The cell lines of human prostate (LNCaP), human breast (MCF-7) and human colon cancer (HCT-116) used in the present study was retrieved from ATCC.

MTT Assay: The water, ethanol, methanol and acetone extracts of *A. calvertii* flowers were screened for their cytotoxic properties against on LNCaP, HCT-116 and MCF-7 cancer cell lines. The cells viability was determined using trypan blue (0.4%). The study was not started when the viability of cells was below 90%. Cells were counted by hemocytometer and $1.5x10^4$ cells were put in all wells. The cells were treated with 1, 5, 10, 25, 50, 75 and 100 µg/mL concentrations of *A. calvertii* flowers extracts in DMSO. They were incubated for 24 h. Effects of the % cell viability of *A. calvertii* flower extracts were evaluated by MTT assay [18,19].

G. STATISTICAL ANALYSES

All the statistical analyses were calculated with SPSS Statistics 22.0 for Windows software. The antiradical results were evaluated using the analysis of variance (ANOVA). For antiproliferative activity tests, normal distribution was obtained using Kolmogorov Smirnov test ($p < 0.05$). The IC_{50} values were calculated by using % cell viabilities of the extracts.

III. RESULT AND DISCUSSION

A. ANTIRADICAL PROPERTIES

The antiradical results of *A. calvertii* flowers are presented in Table 1. In all the antiradical assays (ABTS, OH and DPPH, respectively), trolox (95.76, 94.56, 96.89%, respectively) had the highest scavenging activity among all the extracts. Similarly, Zakizadeh et al. [6] reported that *A. hyrcana* flower extracts were lower scavenged the DPPH radicals than standard antioxidants BHA, ascorbic acid and quercetin. Anlas et al. [20] showed that *A. apterocarpa* roots water extract scavenged the DPPH radicals in ratio of 84.6%. Ertas et al. [21] reported that *A. pallida* acetone extract was higher scavenged ABTS radicals than standard antioxidants tocopherol and BHT.

Table 1. *$ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic contents of Alcea calvertii flower extracts*

Samples	$ABTS^{+\bullet}$ Scavenging (%)	$OH^{\bullet}$ Scavenging (%)	$DPPH^{\bullet}$ Scavenging (%)	Total Flavonoid (*µg CE/g*)	Total Proanthocyanidin (*µg CE/g*)	Total Phenolic (*mg GAE/g*)
A. calvertii (water)	63.64±0.75[b]	83.01±0.25[b]	91.23±0.81[b]	1155.42±3.17	158.56±0.95	65.06±0.81
A. calvertii (ethanol)	51.55±1.03[d]	77.83±0.71[a]	27.68±2.10[d]	1193.45±3.32	731.94±1.84	31.79±0.32
A. calvertii (methanol)	60.42±1.27[b]	72.65±0.39[d]	66.92±0.90[c]	1643.70±3.25	941.85±1.45	55.98±0.78
A. calvertii (acetone)	57.58±0.87[c]	80.10±0.23[c]	19.69±0.77[e]	1113.31±3.85	1045.22±2.56	22.63±0.22
Trolox	95.76±0.39[a]	94.56±0.81[a]	96.89±0.92[a]	-	-	-

Within a column, different superscript letters are significantly different at $p<0.001$. The antiradical activity results were calculated for 500 *µg/mL* extract concentrations. Total flavonoid and total proanthocyanidin contents were expressed as µg catechin equivalent/g extract, and total phenolic content were expressed as mg gallic acid equivalent/g extract.

B. PHYTOCHEMICAL RESULTS

TPAC, TFC and TPC of *A. calvertii* flowers are summarized in Table 1. *A. calvertii* flower water, ethanol, methanol and acetone extracts of TFC were 1155.42, 1193.45, 1643.70, and 1113.31 µg CE/g extract, respectively; TPAC were 158.56, 731.94, 941.85 and 1045.22 µg CE/g extract, respectively; TPC were 65.06, 31.79, 55.98 and 22.63 mg GAE/g extract, respectively. Zakizadeh et al. [6] determined that there were 8.1 mg GAE/g, 68.9 mg GAE/g and 14.7 mg GAE/g extract of the total phenolic compounds in the extracts of *A. hyrcana* leaf, flower and seeds, respectively.

The composition of phenolic acids, non-flavonoid and flavonoids in *A. calvertii* flower is presented in Table 2. Quercetin, morin and resveratrol were detected. The catechin (63.15 µg/g) was the dominant component. The gallic, vanillic, ferulic, caffeic, hydroxycinnamic and rosmarinic acids were detected as the phenolic acids. Gallic acid (1674.10 µg/g) and vanillic acid (1659.05 µg/g) were the major component.

The vitamins, phytosterols and fatty acids in *A. calvertii* flower is shown in Table 2. Ertas et al. [21] determined that *A. pallida* extracts were included 31.2% 16:0, 7.3% 18:0, 11.2% 18:1, 10.2% 18:2, 15.9% 18:3; *A. apterocarpa* extracts were included 21.7% 16:0, 5.1% 18:0, 24.8% 18:1, 25.6% 18:2, 6.9% 18:3.

There were 24.3 mg quercetin equivalent (QE)/g, 24.7 mg QE/g and 28.3 mg QE/g extract of the total flavonoid in the extracts of *A. hyrcana* leaf, flower and seeds (respectively) determined by Zakizadeh et al. [6]. Anlas et al. [20] showed that *A. apterocarpa* root ethanol extracts were contained 33.28 mg GAE/g extract total phenolic contents. Ertas et al. [21] determined that *A. pallida* acetone, methanol and water extracts were included 175.00 µg Pyrocatechol Equivalent (PE)/mg extract, 106.51 µg PE/mg extract, 152.74 µg PE/mg extract total phenolic compounds (respectively) and 19.66 µg Quercetin Equivalent (QE)/mg extract, 13.30 µg QE/mg extract, 12.19 µg QE/mg extract total flavonoid contents (respectively). In the same study, it was calculated that *A. apterocarpa* acetone, methanol and water extracts were contained 144.18 µg PE/mg extract, 96.23 µg PE/mg extract, 109.93 µg PE/mg extract total phenolic compounds (respectively) and 18.71 µg QE/mg extract, 10.94 µg QE/mg extract, 11.47 µg QE/mg extract total flavonoid contents (respectively) [21]. According to these results, the different results reported in the present study can be due to the different species studied.

Table 2. *Contents and composition of flavonoids, non-flavonoid, phenolic acids, vitamins, phytosterols and fatty acids in Alcea calvertii flowers*

Flavonoids, Non-Flavonoids and Phenolic Acids	**(*µg/g*)**
Morin	0.15±0.01
Quercetin	6.80±0.55
Catechin	63.15±1.30
Resveratrol	13.85±0.75
Vanillic Acid	1659.05±3.30
Gallic Acid	1674.10±2.95
Hydroxycinnamic Acid	8.25±0.45
Caffeic Acid	164.55±1.20
Ferulic Acid	1137.30±2.95
Rosmarinic Acid	42.90±0.75
Vitamin and Phytosterols	**(*µg/g*)**
Retinol	0.03±0.00
δ-Tocopherol	1.45±0.15
α-Tocopherol	0.40±0.05
Vitamin K	0.50±0.05
Vitamin D	19.75±1.05
Ergosterol	27.10±1.15
Stigmasterol	496.95±3.75
Fatty Acids (FA)	**(%)**
16:0	27.03±0.22
16:1	8.57±0.52
18:0	8.76±0.44
18:1	12.53±0.48
18:2	28.77±0.91
18:3	14.34±0.52
Saturated FA	35.79
Unsaturated FA	64.21

C. ANTIMICROBIAL RESULTS

The antimicrobial results of *A. calvertii* flowers are shown in Table 3. *A. calvertii* flower methanol extract better inhibit the growth of *L. monocytogenes*, *E. coli*, *B. subtilis* and *C. albicans* than nystatin and streptomycin sulfate. In the previous studies, *A. pallida* and *A. apterocarpa* methanol and acetone extracts inhibit the growth of *S. aureus*, *E. coli*, *P. aeruginosa*, *Streptococcus pyogenes* and *C. albicans* [21]; *A. apterocarpa* seeds methanol extracts inhibit the growth of *P. aeruginosa* [22]; *A. rosea* ethanol extracts inhibit growth of *Bacillus anthracis*, *B. cereus*, *S. aureus*, *S. epidermidis*, *S. pyogenes*, *Salmonella typhi*, *K. pneumoniae* and *P. aeruginosa* [23]; *A. rosea* flowers ethanol, methanol and water extracts inhibit the growth of *E. coli*, *S. aureus*, *S. epidermidis*, *Salmonella typhimirium*, *P. aeruginosa* microorganisms [24]. These results showed that *Alcea* species have great antimicrobial potential against to above mentioned microorganisms.

Table 3. *The antimicrobial activities of Alcea calvertii flower extracts (mm zone)*

Microorganisms	*A.calvertii* (water)	*A.calvertii* (ethanol)	*A.calvertii* (methanol)	*A.calvertii* (acetone)	Standard
Escherichia coli	8	9	10	9	10
Proteus vulgaris	nd	nd	nd	nd	10
Pseudomonas aeruginosa	nd	nd	nd	nd	15
Listeria monocytogenes	8	9	9	9	8
Klebsiella pneumoniae	nd	nd	nd	nd	9
Bacillus subtilis	9	9	10	10	9
Bacillus megaterium	8	9	9	9	12
Staphylococcus aureus	8	10	10	9	12
Candida albicans	8	10	11	8	10

Streptomycin sulfate (10 mg/disc) and Nystatin (30 mg/disc) were used as standard antibiotic discs. The diameter of the paper discs was 6 mm.
nd: not determined

D. ANTIPROLIFERATIVE RESULTS

IC_{50} values of *A. calvertii* extracts are showed in Table 4. According to these results, *A. calvertii* flowers water, ethanol, methanol and acetone extracts were showed highly antiproliferative activity on the MCF-7, LNCaP and HCT-116 cancer cell lines at 24 h. It was not found any study about antiproliferative activities of *A. calvertii*. But, when evaluated the antiproliferative activities of *Alcea* genus, Abdel-Salam et al. [25] determined that the flavonoids obtained from ethanol extracts of *A. rosea* were showed antiproliferative activity on against to human liver cancer cell lines (HepG-2). Yaglioglu et al. [26] indicated that *A. rosea* flower extracts have high antiproliferative activity on the human cervical cancer (HeLa) and rat brain tumor (C6) and cell lines.

Table 4. *The IC_{50} values of A. calvertii flower extracts against MCF-7, LNCaP and HCT-116 cancer cell lines for the antiproliferative activity assay*

Samples (*µg/mL*)	MCF-7	LNCaP	HCT-116
A. calvertii water	15.25	11.84	10.78
A. calvertii ethanol	16.16	10.81	12.42
A. calvertii methanol	25.17	9.89	20.08
A. calvertii acetone	16.49	10.40	11.81

IV. CONCLUSIONS

This study aimed to evaluate the antiradical activities, phytochemical compositions, antimicrobial activities and antiproliferative activities of the water, ethanol, methanol and acetone extracts of endemic *A. calvertii* flowers. The as obtained results of this study indicate that *A. calvertii* flowers have antiradical, antimicrobial and antiproliferative activities against to all the above-mentioned radicals, microorganisms and cancer cell lines.

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