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# *In vitro* Antimicrobial, Anticancer and Antioxidant Activities and Bioactive Contents of Endemic *Acanthus dioscoridis* L. var. *dioscoridis* Flowers from Türkiye

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**ABSTRACT**

*A. dioscoridis* is a member of the Acanthaceae family and is represented by approximately 30 species. In Türkiye, 8 species, 6 of which are endemic, are distributed in Eastern and Central Anatolia. In the presented study, the antimicrobial, antiradical, and anticancer properties of flower extracts of endemic *A. dioscoridis* L. var. *dioscoridis* were investigated for the first time. The antiradical activity and phytochemical contents of this plant were also investigated. According to our study results, endemic *A. dioscoridis* flowers extract shows anticancer activity against MCF-7, HCT-116 and LNCaP cancer cell lines, high antiradical activity against ABTS radicals, and effective antimicrobial activity against some microorganism-caused infection in humans. In conclusion, this study can be the first report about the anticancer, antiradical, and antimicrobial properties of endemic *A. dioscoridis* L. var. *dioscoridis* flower extracts.

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## 1. Introduction

Plants are being studied in today's scientific world as a remedy for many diseases thanks to the compounds they contain, such as vitamins, fatty acids, steroids, ellagitannins, flavonoids, saponins, alkaloids and phenolics, which have significant positive effects on human health. It has been proven by many studies that these compound groups and/or compounds belonging to these groups have antioxidant, antiradical, anticancer, antitumor, antimicrobial, antibacterial, etc. effects. When examined specifically in terms of compound groups, stilbenoids and polyphenolic compounds containing various reactive groups in their structures also have these properties. Reactive oxygen species (ROS), such as OH radicals, are spontaneously formed during metabolic processes in living cells and play an essential role in many biochemical events, including oxidative stress. Antioxidant compounds contained in plants can protect living cells from oxygen-related damage. If antioxidants do not eliminate free radicals and their derivatives, oxidative stress can cause many diseases, including cancer [1-3]. Plants used/consumed by humans as food and medicine throughout history are the focus of many studies on anticancer, antioxidant, antiradical, antimicrobial, etc. [4-7].

Acanthaceae is a large plant family with 250 genera and 2700 species distributed from Africa to Southeast Asia. *Acanthus* L. is a member of this family and is represented by approximately 30 species. In Türkiye, 8 species, 6 of which are endemic, are widespread in Eastern and Central Anatolia. Many *Acanthus* species have been widely used in traditional medicine, especially in the Far East, for many years in treating certain diseases such as hepatitis, asthma and lymphoma. It is known that the leaves of the *A.*

*dioscoridis* L. var. *dioscoridis* are used as an expectorant and wound healing agent among the public in our country [8]. In previous studies, it was observed that the antiradical properties of *A. hirsutus* [9], and the anti-inflammatory [10] and antioxidant [11] properties of *A. ilicifolius* were determined. It was understood that the antiradical and anticancer properties of the aerial parts and leaves of *A. dioscroidis* L. var. *dioscoridis* and their phytochemical contents were determined [3,12]. Many studies show that *Acanthus* species are used as traditional folk medicine in the treatment of diseases such as rheumatism, lymphitis, snakebite, liver disorders, stroke, asthma and abdominal pain [13]. Again, many studies have shown that *Acanthus* species are rich in secondary metabolites, fatty acids, alcohols, sterols and glycosides [14,15].

The aim of this study is to determine the antiradical, antimicrobial, anticancer activities and bioactive compounds of *Acanthus dioscoridis* L. var. *dioscoridis* flowers water, ethanol, methanol and acetone extracts.

## 2. Materials and Methods

### 2.1. Plant Materials and Extraction Procedures

The flowers of endemic *A. dioscoridis* were collected in July 2014 from Sivrice/Elazig in Türkiye. The voucher specimen number is Turkoglu 4901. This specimen was stored in the herbarium of Firat University, Faculty of Science, Department of Biology, Elazig/Türkiye. The flowers were dried at dark and room temperature. Dried flowers were pulverized using a mechanic grinder, and then 20 g of the sample was extracted with 200 mL of solvent (water, ethanol, acetone and methanol). All the extracts were centrifuged. After centrifuging and filtrating of solvents, the supernatant was concentrated. The dried extract was dissolved in DMSO (µg/mL) [4].

### 2.2. Determination of Radical Scavenging Activities (RSAs)

The DPPH, $ABTS^{\bullet+}$ and hydroxyl (OH) radical scavenging activities (RSAs) were determined by the methods of Brand-Williams et al. [16], Re et al. [17] and Halliwell et al. [18], respectively. All tests were repeated three times and the average values were calculated. The radical scavenging activity percentages (RSA%) for each sample were calculated by the following equation:

$RSA\% = [(A_0 - A_1)/A_0] \times 100$

$A_0$: control absorbance; $A_1$: sample absorbance.

### 2.3. Determination of Phytochemical Components

The determination of total phenolic contents (gallic acid used as standard), total flavonoid contents (catechin used as standard), total proanthocyanidin content (catechin used as standard) were performed according to the methods of Slinkard and Singleton [19], Kim et al. [20] and Amaeze et al. [21], respectively. The determination of flavonoid and phenolic acids was performed according to the method of Zu et al. [22] in the *A. dioscoridis* by HPLC. Quercetin, kaempferol, naringenin, resveratrol, vanillic acid, gallic acid, caffeic acid, ferulic acid and rosmarinic acid were quantified in the *A. dioscoridis* flowers by HPLC. Fatty acids in the endemic *A. dioscoridis* flowers were analyzed according to Christie's method [23] by Gas Chromatography (GC). The fatty acids analyses results were expressed as a percent of samples. Lipophylic vitamins and phytosterols were analyzed according to the method of Sánchez-Machado et al. [24] and Lopez-Cervantes et al. [25] by High Performance Liquid Chromatography (HPLC) from the *A. dioscoridis* flowers. The results of the analyses were expressed as µg/g.

### 2.4. Determination of Antimicrobial Properties

*E. coli* ATCC 25922, *B. megaterium* DSM 32, *B. subtilis* IMG 22, *P. vulgaris* FMC 1, *P. aeruginosa* DSM 50071, *L. monocytogenes* SCOTTA, *K. pneumoniae* FMC 5, *S. aureus* COWAN 1 bacteria and *C. albicans* FMC 17 fungus were used as test organisms. The antimicrobial activity tests were performed according to Collins and Lyne's method [26] by the disc diffusion method. Streptomycin sulfate (10 mg/disc) was used as the standard antibiotic for the bacteria, and Nystatin (30 mg/disc) was used as the standard antibiotic for fungus.

### 2.5. Determination of Anticancer Properties

#### 2.5.1. Cell Culture

The cell lines of MCF-7 (human breast cancer), HCT-116 (human colon cancer), and LNCaP (human prostate cancer) were used the anticancer studies. These cells were retrieved from American Type Culture Collection (ATCC).

#### 2.5.2. MTT Test

*A. dioscoridis* extracts (water, acetone, methanol and ethanol) were studied for anticancer activity against to the LNCaP, HCT-116 and MCF-7 cell lines. The viability of the cells was determined using 0.4% trypan blue. Effects of the % cell viability of extracts were evaluated by the MTT test [27,28].

### 2.6. Statistical analyses

The anticancer activity results were evaluated using the Kolmogorov Smirnov test ($p<0.05$); antiradical activity tests were evaluated using Duncan's multiple range test (DMRT) and the analysis of variance (ANOVA) by the SPSS Statistics 22.0 software. The $IC_{50}$ values were calculated by using % cell viabilities of extracts.

## 3. Results and Discussion

### 3.1. Antiradical Properties

The antiradical properties results of *A. dioscoridis* flowers extracts are presented in Table 1. *A. dioscoridis* water and methanol (%98.03, %98.56, respectively) extracts were exhibited higher ABTS radical scavenging activity than standard antioxidant BHA (97.59%). For the OH radical scavenging test, *A. dioscoridis* water extract (98.79%) was showed higher scavenging activity than standard antioxidant BHA (96.77%). For the DPPH radical scavenging test, standard antioxidant BHA (96.55%) had the highest radical scavenging activity among all the extracts.

**Table 1.** $ABTS^{+\bullet}$, $OH^{\bullet}$, $DPPH^{\bullet}$ radicals scavenging activities, total flavonoid, total proanthocyanidin and total phenolic contents of *Acanthus dioscoridis* flowers 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. dioscoridis* water | 98.03±0.22[a] | 98.79±0.09[a] | 80.33±0.62[c] | 1021.39±2.19 | 390.78±1.05 | 112.34±1.36 |
| *A. dioscoridis* ethanol | 78.45±0.01[b] | 83.26±0.29[b] | 92.64±0.25[b] | 1559.66±2.64 | 366.33±1.42 | 68.78±0.26 |
| *A. dioscoridis* methanol | 98.56±0.22[a] | 96.28±0.44[a] | 93.57±0.01[b] | 3837.98±3.97 | 435.22±1.69 | 104.97±1.31 |
| *A. dioscoridis* acetone | 66.91±1.05[c] | 78.97±0.39[b] | 37.67±0.95[d] | 967.32±1.44 | 401.89±1.34 | 22.38±0.74 |
| BHA | 97.59±0.35[a] | 96.77±0.22[a] | 96.55±0.32[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.

Keskin [12] found that hexane, ethyl acetate and methanol extracts of *A. dioscoridis* aerial parts destroyed DPPH radicals by 14.37%, 52.06% and 85.08%, respectively. When the extracts of methanol, the common solvent in our and his study, were compared (93.57% and 85.08%, respectively), it is thought that the difference is due to the extraction of different parts of the same plant. In another study, Abdullah et al. [3] suggested that methanol extracts of *A. dioscoridis* leaves exhibit DPPH radical scavenging activity.

Also, when antioxidant studies on *Acanthus* genus were examined, Harput et al. [9] showed that *A. hirsutus* water extract had DPPH radical scavenging activity, while Babu et al. [11] showed that *A. ilicifolius* ethanol extract had OH radical scavenging activity. In another study, Kumar et al. [10] determined that *A. ilicifolius* methanol extract had DPPH, ABTS and OH radical scavenging activity. All these results show that species belonging to *Acanthus* genus have antiradical properties.

### 3.2. Phytochemical Composition

The phytochemical contents of *A. dioscoridis* extracts are presented in Table 1 and Table 2. *A. dioscoridis* water, ethanol, methanol and acetone extracts of total flavonoid amounts were 1021.39, 1559.66, 3837.98 and 967.32 µg CE/g extract, respectively; total proanthocyanidin amounts were 390.78, 366.33, 435.22, and 401.89 µg CE/g extract, respectively; total phenolic compounds amounts were 112.34, 68.78, 104.97, and 22.38 mg GAE/g extract, respectively. Flavonoid amounts of *A. dioscoridis* were quercetin (0.03 µg/g), kaempferol (0.50 µg/g), naringenin (3.95 µg/g) and resveratrol (6.45 µg/g); the phenolic acid amounts of *A. dioscoridis* were vanillic acid (433.65 µg/g), gallic acid (6827.85 µg/g), caffeic acid (2997.60 µg/g), ferulic acid (22.00 µg/g) and rosmarinic acid (52.55 µg/g). The lipid-soluble vitamins of *A. dioscoridis* were retinol (0.03 µg/g), α-tocopherol (0.50 µg/g), vitamin K (0.05 µg/g) and vitamin D (0.70 µg/g); the phytosterols of *A. dioscoridis* were ergosterol (18.75 µg/g), stigmasterol (4.35 µg/g). The fatty acids content in *A. dioscoridis* were 30.79% palmitic acid (16:0), 3.55% palmitoleic acid (16:1), 30.85% stearic acid (18:0), 4.94% oleic acid (18:1), 15.50% linoleic acid (18:2), 6.16% linolenic acid (18:3), 8.51% eicosenoic acid (20:1), 61.34% total saturated fatty acids, 38.66% total unsaturated fatty acids.

Keskin [12] showed that hexane, ethyl acetate and methanol extracts of *A. dioscoridis* aerial parts contained 28.16 µg GAE/mg, 33.89 µg GAE/mg and 71.18 µg GAE/mg total phenolic compounds; 34.45 µg QE/mg, 153.54 µg QE/mg and 16.85 µg QE/mg total flavonoids, respectively. In the same study, it was determined that this plant contains 4.09% myrsitic acid (14:0), 0.47% pentadecanoic acid (15:0), 26.04% palmitic acid (16:0), 0.78% heptadecanoic acid (17:0), 6.80% stearic acid (18:0), 1.71% palmitoleic acid (16:1), 8.54% oleic acid (18:1), 16.26% linoleic acid (18:2), 35.25% linolenic acid (18:3), 38.22% total saturated fatty acids, 61.76% total unsaturated fatty acids [12]. The differences in fatty acid, total phenolic compound and total flavonoid contents between this study and our study may be due to the different plant parts (aerial parts, flowers) and different extraction solvents studied.

When studies related to the *Acanthus* genus were examined, Harput et al. [9] determined that *A. hirsutus* water extract contained 65.4 mg GAE/g, while Kumar et al. [10] determined that *A. ilicifolius* methanol extract contained 310 mg QE/g total phenolic compounds.

**Table 2.** Contents and composition of flavonoids, phenolic acids, vitamins, phytosterols and fatty acids in *Acanthus dioscoridis* flowers

| **Flavonoids and Phenolic Acids** | **(µg/g)** |
|---|---|
| Quercetin | 0.03±0.00 |
| Kaempferol | 0.50±0.05 |
| Naringenin | 3.95±0.25 |
| Resveratrol | 6.45±0.30 |
| Vanillic Acid | 433.65±2.25 |
| Gallic Acid | 6827.85±3.75 |
| Caffeic Acid | 2997.60±1.95 |
| Ferulic Acid | 22.00±0.55 |
| Rosmarinic Acid | 52.55±0.80 |
| **Vitamin and Phytosterols** | **(µg/g)** |
| Retinol | 0.03±0.00 |
| α-Tocopherol | 0.50±0.05 |
| Vitamin K | 0.05±0.00 |
| Vitamin D | 0.70±0.05 |
| Ergosterol | 18.75±0.45 |
| Stigmasterol | 4.35±0.10 |
| **Fatty Acids (FA)** | **(%)** |
| 16:0 | 30.49±0.88 |
| 16:1 | 3.55±0.11 |
| 18:0 | 30.85±0.77 |
| 18:1 | 4.94±0.33 |
| 18:2 | 15.50±0.54 |
| 18:3 | 6.16±0.32 |
| 20:1 | 8.51±0.24 |
| Saturated FA | 61.34 |
| Unsaturated FA | 38.66 |

### 3.3. Antimicrobial Properties

The antimicrobial properties of *A. dioscoridis* flowers extracts are presented in Table 3. According to these results, it was observed that *A. dioscoridis* ethanol, methanol and acetone extracts have antimicrobial properties on *E. coli*, *P. vulgaris*, *P. aeruginosa*, *B. subtilis*, *B. megaterium* and *S. aureus* bacteria and *C. albicans* yeast. According to the information we obtained from the literature review, no studies were found on the antimicrobial properties of *A. dioscoridis*.

**Table 3.** The antimicrobial activities of *Acanthus dioscoridis* flowers extracts (mm zone)

| Microorganisms | *A. dioscoridis* water | *A. dioscoridis* ethanol | *A. dioscoridis* methanol | *A. dioscoridis* acetone | Standard Antibiotics |
|---|---|---|---|---|---|
| *Escherichia coli* | nd | 8 | 9 | 8 | 10 |
| *Proteus vulgaris* | 8 | 9 | 10 | 8 | 10 |
| *Pseudomonas aeruginosa* | 8 | 9 | 10 | 8 | 15 |
| *Listeria monocytogenes* | nd | nd | nd | nd | 8 |
| *Klebsiella pneumoniae* | nd | nd | nd | nd | 9 |
| *Bacillus subtilis* | 8 | 9 | 10 | 8 | 9 |
| *Bacillus megaterium* | 9 | 10 | 11 | 9 | 12 |
| *Staphylococcus aureus* | 7 | 10 | 11 | 9 | 12 |
| *Candida albicans* | 8 | 11 | 10 | 8 | 10 |

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

### 3.4. Anticancer Properties

The $IC_{50}$ values of anticancer properties of *A. dioscoridis* extracts on the MCF-7, LNCaP, and HCT-116 cancer cell lines are presented in Table 4 and Figure 1. *A. dioscoridis* acetone extract (1.62 µg/mL) has better anticancer activity for the LNCaP cell lines than all the other extracts; *A. dioscoridis* methanol extract (8.30 µg/mL) has better anticancer activity for the MCF-7 cell lines than all the other extracts; *A. dioscoridis* acetone extract (3.82 µg/mL) has better anticancer activity for the HCT-116 cell lines than all the other extracts. All these results show that *A. dioscoridis* flowers extracts have high anticancer activity. To our best knowledge, there is no report about anticancer activities of *A. dioscoridis* flower extracts. For this reason, the present study can be the first report about the anticancer activities of *A. dioscoridis* flower extracts. Abdullah et al. [3] determined that oils obtained from methanol extracts of *A. dioscoridis* leaves have cytotoxic activity against MCF-7 cancer cells. In another study, Harput et al. [9] showed that *A. hirsutus* water extract had antitumor activity on human laryngeal cancer, human rhabdomyosarcoma and transgenic murine L cell lines.

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

| Samples (µg/mL) | MCF-7 | LNCaP | HCT-116 |
|---|---|---|---|
| *A. dioscoridis* water | 8.90 | 4.66 | 7.68 |
| *A. dioscoridis* ethanol | 10.50 | 4.05 | 7.16 |
| *A. dioscoridis* methanol | 8.30 | 4.79 | 6.67 |
| *A. dioscoridis* acetone | 9.28 | 1.62 | 3.82 |

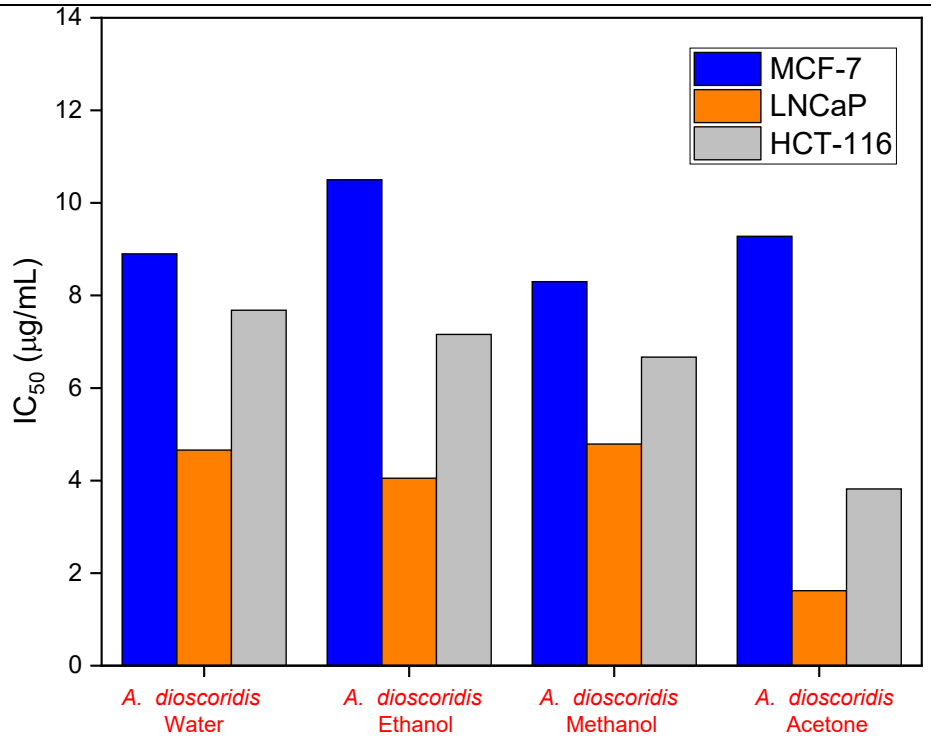


**Figure 1.** The $IC_{50}$ values of *A. dioscoridis* flowers against MCF-7, LNCaP and HCT-116 cancer cell lines

## Conclusion

This study can be first report about the anticancer, antiradical, antimicrobial and phytochemical properties of endemic *A. dioscoridis* L. var. *dioscoridis* flowers extracts. Also, the present work showed that endemic *A. dioscoridis* has antimicrobial, anticancer, antiradical properties and highly bioactive contents, such as phenolic, flavonoids, proanthocyanidins, lipid-soluble vitamins, phytosterols and fatty acids.

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