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ANATOMY AND ANTIOXIDANT CAPACITY ASSESSMENT OF *DIANTHUS ANCYRENSIS*

DIANTHUS ANCYRENSIS'İN ANATOMİSİ VE ANTİOKSİDAN KAPASİTESİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *Dianthus ancyrensis Hausskn. & Bornm. (Caryophyllaceae), commonly known as "Ankara karanfili", is a perennial herbaceous plant characterized by a slender stem and linear leaves. Dianthus species have a long history of traditional uses for various ailments including pain relief, stomachaches, heart diseases, and respiratory problems. Scientific research supported by previous studies has revealed the antifungal, antioxidant, anticancer and antibacterial properties of Dianthus species.*

Material and Method: *In this study, the anatomy of the D. ancyrensis was examined by light microscopy and its antioxidant capacity was evaluated by DPPH• and ABTS•$^{+}$ radical scavenging tests. Plant material was collected from Ankara and extracted by maceration in 70% ethanol. Anatomical sections were prepared and visualised under the microscope with Sartur reagent, gallic acid and trolox were used as references in the antioxidant analyses. Total phenolic content and IC_{50} values were calculated spectrophotometrically.*

Result and Discussion: *Anatomical observations revealed a disk-shaped stem with stomata and simple covering hairs present on the epidermal layer, accompanied by 2 or 3 subsidiary cells. The leaf displayed centric characteristics, with a main vascular bundle accompanied by two smaller veins. Stomata were located on the upper epidermis, while simple covering hairs were prominent on the midrib and leaf margin. The midrib exhibited sclerenchymatous tissue surrounding the phloem from the abaxial side, and large crystal druses were observed in both the midrib and mesophyll. The total phenolic content of the extract was measured at 216.97 mg GAE/g of dry extract. DPPH• and ABTS•$^{+}$ radical scavenging capacities of the extract were calculated as 2.68 and 1.68 mg/ml, respectively.*

Keywords: *Antioxidant, caryophyllaceae, Dianthus, Dianthus ancyrensis, pharmaceutical botany, plant anatomy*

ÖZ

Amaç: *Dianthus ancyrensis Hausskn. & Bornm. (Caryophyllaceae), halk arasında "Ankara karanfili" olarak bilinen, ince gövdeli ve linear yapraklı çok yıllık otsu bir bitkidir. Dianthus türlerinin ağrı kesici, mide ağrıları, kalp hastalıkları ve solunum problemleri gibi çeşitli*

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rahatsızlıklar için geleneksel kullanımları uzun bir geçmişe sahiptir. Önceki çalışmalarla desteklenen bilimsel araştırmalar, Dianthus türlerinin antifungal, antioksidan, antikanser ve antibakteriyel özelliklerini ortaya çıkarmıştır.

Gereç ve Yöntem: *Bu çalışmada, D. ancyrensis bitkisinin anatomisi ışık mikroskobu ile incelenmiş, antioksidan kapasitesi ise DPPH• ve ABTS•$^{+}$ radikal temizleme testleri ile değerlendirilmiştir. Bitki materyali Ankara'dan toplanmış ve %70 etanolde maserasyon yöntemiyle ekstre edilmiştir. Anatomik kesitler mikroskopta Sartur reaktifi ile hazırlanarak görüntülenmiş, antioksidan analizlerde galik asit ve trolox referans olarak kullanılmıştır. Toplam fenolik içerik ve IC_{50} değerleri spektrofotometrik olarak hesaplanmıştır.*

Sonuç ve Tartışma: *Anatomik incelemelerde disk şeklinde olan gövde epidermasında 2 veya 3 yardımcı hücrenin eşlik ettiği stomalar ve basit örtü tüyleri gözlendi. Yaprak merkezi olup, yaprakta iki küçük iletim demetinin eşlik ettiği bir ana iletim demeti mevcuttur. Stomalar üst epidermada bulunurken, orta damar ve yaprak kenarlarında basit örtü tüyleri belirgindir. Orta damar, abaksiyal taraftan floemi çevreleyen sklerenkimatik bir doku içerir ve hem orta damar hem de mezofilde büyük druzlar gözlenmiştir. Ekstrenin toplam fenolik içeriği 216.97 mg GAE/g kuru ekstre olarak ölçüldü. Ekstrenin DPPH• ve ABTS•$^{+}$ radikal temizleme kapasiteleri sırasıyla 2.68 ve 1.68 mg/ml olarak hesaplandı.*

Anahtar Kelimeler: *Antioksidan, bitki anatomisi, caryophyllaceae, Dianthus, Dianthus ancyrensis, farmasötik botanik*

INTRODUCTION

Caryophyllaceae is a large family that grows in temperate climate zones and is widely distributed along the Mediterranean coast and in Asia. However, some species of the family are also common throughout the world. The Caryophyllaceae family, which includes approximately 102 genera and more than 2500 species, is especially common in the Mediterranean and Irano-Turanian regions [1,2]. *Dianthus* L. has a wide distribution area from Europe to the temperate zone. Plants belonging to this genus also grow naturally in Asia and the Himalayas, Africa, and Arctic America [2,3]. The genus has 356 accepted species [2]. *Dianthus* species is known in many traditional uses in pain reliever, calmative, stomachache, heart disease, antitussive, diuretic, diaphoretic, stimulant, genital warts, toothache, common cold, sedative, digestive, headache, asthma, liver and kidney problems [3,4]. Previous studies on *Dianthus* species have shown that these species have antifungal, antioxidant, anticancer and antibacterial effects [3-5].

Reactive oxygen species (ROS), partially reduced metabolites of oxygen, are molecules that have a broad biological effect in cells. ROS play an important role in regulating cellular redox homeostasis and signal transduction. At high concentrations, ROS cause cell damage and pathological processes, while at low concentrations they contribute to normal physiological processes by modulating cellular signaling. These ROS arise as a byproduct of cell metabolism, but they can also be produced by specific plasma membrane oxidases upon stimulation of growth factors and cytokines [6,7]. Oxidative stress is associated with the development processes of diseases such as cardiovascular, neurodegenerative and cancer. However, there are many studies that prevent free radicals from damaging cells due to the antioxidant properties of some secondary compound groups of plants [8-11]. It is very important to make correct diagnosis and identification of plants that have medicinal uses and have potential as raw materials for herbal medicine, such as *Dianthus* species. The light microscopy analysis is the most effective and practical method that can be used to elucidate the anatomical structures of plants and to diagnose them correctly [12-14].

Dianthus ancyrensis Hausskn. & Bornm., popularly known as "Ankara karanfili", is a herbaceous plant [15]. The plant is perennial with a thin stem and has linear leaves. The inflorescence is subcapitate or with only 1-2 flowers. The petals are pink and ovate, and the flowering period is June-August [16]. In this study, the anatomical structure of endemic *D. ancyrensis* was illuminated by light microscopy and its antioxidant potential was determined by radical scavenging capacity determination.

MATERIAL AND METHOD

Plant Materials

The plant materials were collected from Beynam Forest (Ankara/Türkiye). A plant sample was deposited in the Ankara University Faculty of Pharmacy Herbarium (AEF 30850).

Light Microscopy Analysis

For anatomical studies, samples were stored in 70% alcohol. The surface and transverse sections of these samples were converted into microscopic preparation using a razor blade. For microscopic examinations, *Sartur Solution* [17] was used. The microphotographs were obtained with a Leica DM 4000B integrated microscope [12-14].

Extraction Processes

The ethanolic extracts were prepared using the maceration method. 10 grams were taken from the aerial parts of the plant and extraction was started with 70% ethanol. The powdered plant parts were macerated for 24 hours at room temperature, using 100 ml of 70% ethanol each time. After the obtained ethanolic extracts were passed through filter paper, they were concentrated and dried in a rotavapor under low pressure [9].

Determination of Total Phenolic Content and Free-Radical Scavenging Assay

The aqueous solutions of the plant extract were prepared and 100 µl of these solutions were taken and treated with 400 µl (v/v) Folin Ciocalteu reagent. 800 µl of 5% sodium carbonate solution was added to the mixture. The tubes were then incubated at room temperature for 30 minutes. After incubation, absorbance was measured at 765 nm. Each experiment was performed in 3 replicates. The total phenolic content of the extract was calculated from a standard calibration curve prepared with gallic acid. The total amount of phenol in the samples was calculated as mg gallic acid equivalent per gram of dry extract (mg GAE/g extract) [18].

100 µM DPPH• solution was prepared using methanol. DPPH• solutions were treated with different concentrations of plant extracts. Final extract concentrations were prepared as 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/ml. The prepared solution was incubated at room temperature for 30 minutes. Then, spectrophotometric absorbance measurements were made at 517 nm. Gallic acid was used as reference material [19].

7 mM $ABTS^{\bullet+}$ was first dissolved in water and reacted with 2.45 mM potassium persulfate. The resulting radical solution was kept in the dark at room temperature for approximately 12-16 hours. Then, $ABTS^{\bullet+}$ working solution was prepared by diluting the solution with ethanol. $ABTS^{\bullet+}$ solutions were treated with different concentrations of plant extracts. Final extract concentrations were calculated as 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/ml. After the prepared solution was kept at room temperature for 6 minutes, absorbance spectrophotometric measurements were taken at 734 nm. Trolox was used as reference material [18].

The free radical scavenging capacities of the extracts were calculated using the following formula:

$$\% \text{ inhibition} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})/\text{Absorbance}_{\text{Control}}]x100$$

A standard curve was created for the extract using % inhibition values for various concentrations of the plant extract and the half maximum inhibitory concentration (IC_{50}) was calculated.

Statistical Analysis

Each test was conducted three times, and the average value and standard deviation (SD) were calculated. Statistical analyses were carried out with SPSS V23.0. Statistical significance was determined using one-way analysis of variance (ANOVA) with a significance threshold of $p \leq 0.05$.

RESULT AND DISCUSSION

Anatomical Features

The transverse section of the stem was observed as Figure 1. The transverse section of the stem is disk-shaped. The epidermal layer contains unicellular simple covering hairs. The epidermal layer is covered with a thick cuticle. The epidermal cells are single-layered, thin-walled. The cells of epidermal layer are square-rectangular and sometimes their outer walls are domed. The cortex consists of 3-8 rows of thin-walled epidermal cells and contains starch. The endodermis occasionally contains crystal druses in enlarged parenchymatic cells. The vascular bundle is surrounded by a pericyclic sclerenchymatous ring. The pith cells are thin-walled, rounded, parenchymatous cells and intracellular spaces are large. Sometimes, the pith cell walls are melted, resulting in the formation of a hollow in the pith area.

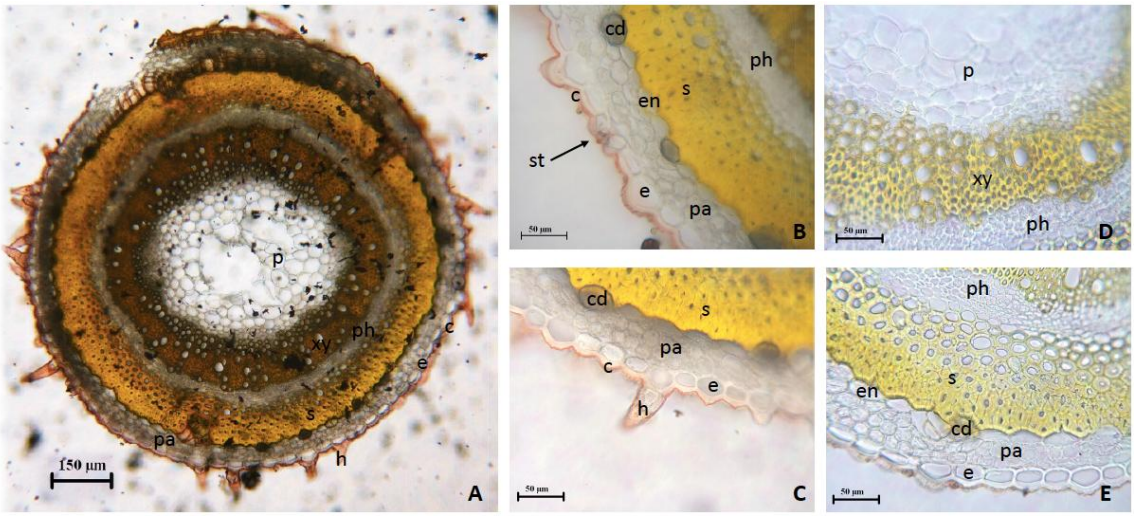


Figure 1. Transverse section of the stem. c: cuticle, cd: crystal druse, e: epidermis, en: endodermis, h: hair, p: pith, pa: cortex parenchyma, ph: phloem, s: sclerenchyma, st: stomata, xy: xylem

In the surface section of the stem, the epidermal cells were observed as thin-walled and mostly elongated rectangular in shape. The simple covering hairs are localized in the epidermis. Additionally, the stomata are observed in the epidermal layer. The stomata are accompanied by 2 or 3 subsidiary cells (Figure 2).



Figure 2. Surface section of the stem. h: hair, st: stomata

The leaf is centric. In the cross section of the leaf, it was observed that the leaf has one main vascular bundle accompanied by two smaller ones (Figure 3A). Especially the outer walls of the leaf

epidermal layer cells showed thickening. The epidermal layer is also covered by thick cuticle. The stomata are located on the upper epidermis. The simple cover hairs are located especially on the midrib and on the margin of the leaf. In the midrib of the leaf, the lower epidermal layer is domed outwards. The upper epidermal layer partially protrudes outward. In the midrib, the phloem is surrounded by sclerenchymatous tissue from the abaxial side. This tends to completely cover the vascular bundle. Large crystal druses are observed in the midrib (Figure 3B). The mesophyll layer is filled with undifferentiated palisade cells. This layer consists of thin-walled, elongated and crushed palisade cells. It also contains large crystal druses (Figure 3C).

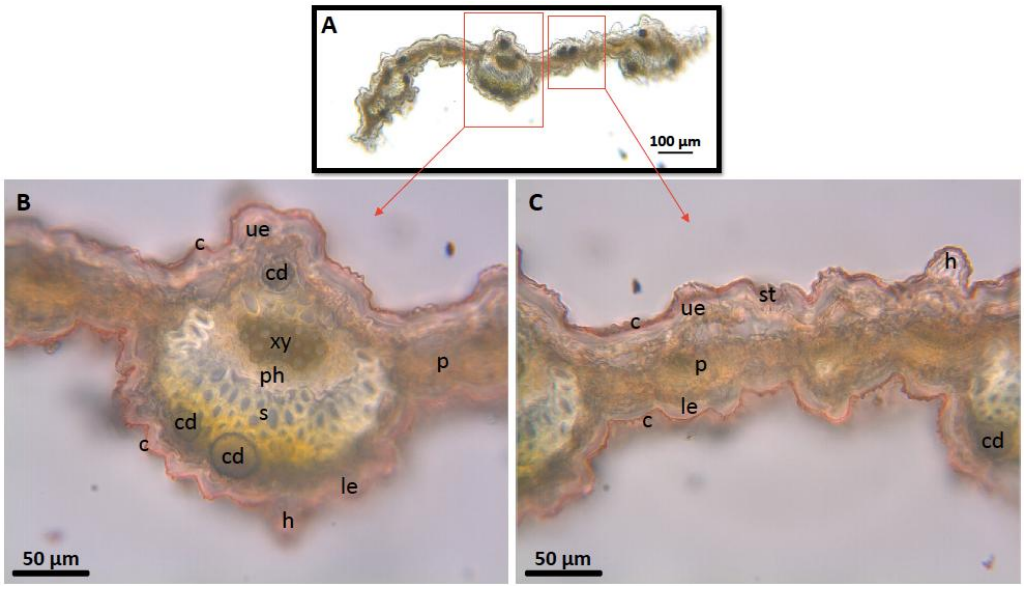


Figure 3. Transverse section of the leaf. A: general view, B: midrib, C: mesophyll, c: cuticle, cd: crystal druse, h: hair, le: lower epidermis, p: parenchyma, ph: phloem, s: sclerenchyma, st: stomata, ue: upper epidermis, xy: xylem

In the surface section of the leaf, stomata and covering hairs were observed in the upper epidermal layer. The stomata are accompanied by 2 or 3 subsidiary cells (Figure 4). The simple cover hairs are located especially in the midrib and at the margin of the leaf.



Figure 4. Surface section of the leaf. st: stomata

Antioxidant Capacity

The percentage (%) yield of the obtained ethanol extract was calculated as 10.45. The total phenolic content of the plant extract was determined as 216.97 mg GAE/g dry extract. The DPPH• radical scavenging capacity (IC_{50}) of the extract was calculated as 2.68 mg/ml compared to gallic acid (1.51 mg/ml). ABTS•$^{+}$ radical scavenging capacity (IC_{50}) was calculated as 2.50 mg/ml compared to Trolox (1.68 mg/ml) (Table 1).

Table 1. Total phenolic content and free radical scavenging capacity results

	Total Phenolic content (mg gallic acid/g extract)±SD*	**DPPH IC_{50} value (mg/ml)±SD***	**ABTS IC_{50} value (mg/ml)±SD***
Ethanol extract	216.97±1.16	2.68±0.03	2.50±0.03
Gallic acid	-	1.51±0.02	-
Trolox	-	-	1.68±0.02

*SD: Standard deviation

In this study, the anatomical structure of endemic *Dianthus ancyrensis* and the antioxidant capacity of the ethanolic extract of aerial parts were investigated. The anatomical results showed that the stem was disk-shaped. The stomata and simple covering hairs were observed on the epidermal layer. The stomata are accompanied by 2 or 3 subsidiary cells. The endodermis occasionally contains crystal druses in enlarged parenchymatic cells. The vascular bundle is surrounded by a pericyclic sclerenchymatous ring. The pith cell walls are melted, resulting in the formation of a hollow in the pith area. The anatomical features of the plant showed that the leaf was a centric leaf. A main vascular bundle accompanied by two smaller veins is the common characteristic feature of the leaf. The stomata are located on the upper epidermis. The simple covering hairs are located especially on the midrib and on the margin of the leaf. In the midrib, the phloem is surrounded by sclerenchymatous tissue from the abaxial side. Large crystal druses are observed in both the midrib and the mesophyll. The stomata and covering hairs were observed on the upper epidermal layer of leaf. The lower epidermal layer contains only hairs, not stomata.

Metcalfe and Chalk (1965) [20] stated that the plants of the *Dianthus* genus have simple, unicellular covering hairs. They also reported that stomata are generally of the caryophyllaceous type and being accompanied by 2 subsidiary cells. They also noted that stomata sometimes tending to be of the cruciferous type. The results of this study are consistent with the previous study. In addition, other anatomical markers compatible with the general anatomical features of the family in the previous reports were also identified in this study. The anatomical features such as centric leaves, large crystal druses, and pericyclic sclerenchymatous ring are the taxonomic markers compatible with the previous reports [20-22]. In this study, the total phenolic content and antioxidant capacity of the ethanolic extract obtained from the aerial parts of the plant were performed by radical scavenging tests. According to results, the total phenolic content of the plant extract was determined as 216.97 mg GAE/g dry extract. The DPPH• radical scavenging capacity of the extract was calculated as IC_{50}: 2.68 mg/ml compared to gallic acid and ABTS•$^{+}$ radical scavenging capacity was calculated as IC_{50}: 2.50 mg/ml compared to trolox. Previous studies reported the total phenolic content of *D. ancyrensis* as 28.41 mg GAE/g dry extract in flower methanol extracts and 33.94 mg GAE/g dry extract in leaf+stem methanol extract. In the DPPH• test results, flower and leaf+stem extracts were reported as 5.14 mg/ml and 2.23 mg/ml, respectively [3].

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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