T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

DETERMINATION OF BIOLOGICAL ACTIVITIES AND PHENOLIC CONSTITUENTS OF ENDEMIC *DIANTHUS* SPECIES IN BOLU

MASTER OF SCIENCE

AYŞE ÇELİK

ACADEMIC SUPERVISOR
Prof. Dr. Arzu TÜRKER

BOLU, JUNE - 2024

APPROVAL OF THE THESIS

DETERMINATION OF BIOLOGICAL ACTIVITIES AND PHENOLIC CONSTITUENTS OF ENDEMIC *DIANTHUS* SPECIES IN BOLU submitted by **Ayşe ÇELİK** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 28.06.2024** by

Examining Committee Members	**Signature**
Supervisor Prof. Dr. Arzu TÜRKER Bolu Abant Izzet Baysal University	....
Member Prof. Dr. Necmi AKSOY Düzce University	....
Member Assoc. Prof. Dr. Hamdi ZENGİNBAL Bolu Abant Izzet Baysal University	....

Prof. Dr. İbrahim KÜRTÜL
Director of Instıtute of Graduate Studies

ETHICAL DECLARATION

In this thesis dissertation that was properly prepared according to the Thesis Writing Rules of Bolu Abant İzzet Baysal University of the Institute of Graduates Studies, I hereby declare that;

- All data, information, and documents presented in the thesis were obtained in accordance with the academic and ethical rules,
- All data, documents, assessments, and results were presented in accordance with the scientific ethical and moral rules,
- All works that were benefitted in the thesis were appropriately cited,
- No alteration was made in the data used,
- Study presented in this thesis is original,

Otherwise, I declare that I accept the loss of all my rights in case any contradiction that may arise against me.

The similarity rate obtained by using the Turnitin program, a plagiarism detection software, is within the limits approved by University Senate.

Ayşe ÇELİK

To my lovely sons Kerem and Kemal

ABSTRACT

DETERMINATION OF BIOLOGICAL ACTIVITIES AND PHENOLIC CONSTITUENTS OF ENDEMIC *DIANTHUS* SPECIES IN BOLU
MSC THESIS
AYŞE ÇELİK
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. ARZU TURKER)

BOLU, JUNE 2024
xiii + 50

Caryophyllaceae family includes *Dianthus* genus, which has over 300 species. Traditional medicine uses a wide variety of *Dianthus* spp. to treat a wide range of ailments, including menostasis, gonorrhea, liver problems, cough, carbuncles, and some types of cancer. Methanolic extracts derived from two distinct parts [(FC - flower+capitulum (1) and LS - leaf+stem (2)] were investigated in order to reveal antibacterial and antioxidant properties of seven distinct endemic *Dianthus* spp. [(*Dianthus ancyrensis* (DAN), *Dianthus aticii* (DAT), *Dianthus balansae* (DBA), *Dianthus carmelitarum* (DCA), *Dianthus kastemboluensis* (DKA), *Dianthus lydus* (DLY), and *Dianthus leucocephalus* (DLE)] found in Bolu, Turkey. DPPH radical scavenging activity, total phenol-flavonoid content, antibacterial activity by disc diffusion method and individual phenolic content by Ultra-High-Performance Liquid Chromatography (U-HPLC) system were performed to understand the medicinal value of these endemic species. Only four (*S. epidermidis*, *S. aureus*, *S. pyogenes*, and *K. pneumonia*) of tested seventeen bacteria showed sensitivity to five different *Dianthus* species. While DLE exhibited modest antibacterial action against *S. epidermidis*, DKA, DAT, and DLY showed substantial susceptibility to *S. pyogenes*. The strongest ability of DBA2 to scavenge radicals was shown to be connected with its highest total phenolic content. DAT2 was the species with the maximum total flavonoid content. The main constituents of endemic *Dianthus* spp. were found to be rutin, phloridzin, ferulic acid, and chlorogenic acid, based on the findings of the U-HPLC analyses These findings emphasize the potential use of seven endemic *Dianthus* spp. as natural resources in the pharmaceutical and nutraceutical industries.

KEYWORDS: Antibacterial; Antioxidant; *Dianthus;* Endemic; Flavonoid; HPLC; Phenol

ÖZET

BOLU'DA YETİŞEN ENDEMİK *DİANTHUS* TÜRLERİNİN BİYOLOJİK AKTİVİTELERİNİN VE FENOLİK İÇERİKLERİNİN BELİRLENMESİ
YÜKSEK LİSANS TEZİ
AYŞE ÇELİK
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI
(TEZ DANIŞMANI: PROF. DR. ARZU TÜRKER)

BOLU, HAZİRAN - 2024
xiii + 50

Caryophyllaceae ailesi içerisinde yer alan *Dianthus* cinsi, 300'den fazla türü içerir. Geleneksel tıpta menostaz, gonore, karaciğer sorunları, öksürük, çıban ve bazı kanser türleri dahil olmak üzere geniş bir hastalık yelpazesini tedavi etmek için çeşitli *Dianthus* türleri kullanır. Bolu, Türkiye'de bulunan yedi farklı endemik *Dianthus* türünün [(*Dianthus ancyrensis* (DAN), *Dianthus aticii* (DAT), *Dianthus balansae* (DBA), *Dianthus carmelitarum* (DCA), *Dianthus kastemboluensis* (DKA), *Dianthus lydus* (DLY) ve *Dianthus leucocephalus* (DLE)] antibakteriyel ve antioksidan özelliklerini daha iyi anlamak için, iki farklı kısımdan [FC - çiçek+baş (1) ve LS - yaprak+sap (2)] elde edilen metanol ekstraktları incelenmiştir. Bu endemik türlerin tıbbi değerini anlamak için DPPH radikal temizleme aktivitesi, toplam fenol-flavonoid içeriği, disk difüzyon yöntemi ile antibakteriyel aktivite ve bireysel fenolik içerik Ultra Yüksek Performanslı Sıvı Kromatografisi (U-HPLC) sistemi kullanılarak analiz edilmiştir. Test edilen on yedi bakteriden sadece dördü (*S. epidermidis*, *S. aureus*, *S. pyogenes* ve *K. pneumoniae*) beş farklı *Dianthus* türüne karşı duyarlılık göstermiştir. DLE, *S. epidermidis*'e karşı orta düzeyde bir antibakteriyel etki sergilerken, DKA, DAT ve DLY, *S. pyogenes*'e karşı önemli bir duyarlılık göstermiştir. DBA2'nin en yüksek toplam fenolik içeriğinin en güçlü radikal süpürme kapasitesi ile ilişkili olduğu bulunmuştur. DAT2 ise maksimum toplam flavonoid içeriğine sahip türdür. U-HPLC analizi bulgularına göre, endemik *Dianthus* türlerinin ana bileşenleri rutin, floridzin, ferulik asit ve klorogenik asit olarak tespit edilmiştir. Bu bulgular, yedi endemik *Dianthus* türünün, ilaç ve nutrasötik endüstrilerde doğal kaynaklar olarak potansiyel kullanımını vurgulamaktadır.

ANAHTAR KELİMELER: Antibakteriyel; Antioksidan; *Dianthus*, Endemik; Flavonoid; HPLC; Fenol

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

$AlCl_3$	**:** Aluminium chloride
ANOVA	**:** Analysis of Variance
DPPH	**:** 2,2-Diphenyl-1-picrylhydrazyl
GAE	**:** Gallic acid equivalent
MEOH	**:** Methanol
Na_2CO_3	**:** Sodium carbonate
NaNO2	**:** Sodium nitrite
NaOH	**:** Sodium hydroxide
UHPLC	**:** Ultra High Performance Liquid Chromatography
DAN	**:** *Dianthus ancyrensis*
DAT	**:** *Dianthus aticii*
DBA	**:** *Dianthus balansae*
DCA	**:** *Dianthus carmelitarum*
DKA	**:** *Dianthus kastembeluensis*
DLY	**:** *Dianthus lydus*
DLE	**:** *Dianthus leucophaeus*
FC	**:** Flower+Capitulum
LS	**:** Leaf+Stem
QSP	**:** Overall quantity of selected phenolics

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Prof. Dr. Arzu Türker. Her guidance, insightful criticism, and unwavering encouragement were instrumental in shaping this thesis. Prof. Türker's expertise in the field pushed me to constantly improve my research, and her understanding support helped me through moments of self-doubt. I am truly grateful for her mentorship throughout this journey.

My sincere thanks go to Prof. Dr. Hakan Türker for his valuable encouragement, insightful suggestions, and constructive comments. His contributions significantly enhanced the quality of my research.

I would like to extend my heartfelt gratitude to my friends Ayça Çimen and Yavuz Baba, whose unwavering support has been a beacon of strength throughout this journey. Their constant encouragement and steadfast presence have been invaluable to me in every aspect. I am truly grateful for their friendship and support.

I am incredibly grateful to my parents, Telli and Hikmet Kaya, and my siblings for their unwavering love and support throughout my academic journey. Their unconditional belief in me gave me the strength to persevere, especially during challenging times.

A special thank you to Aydın Çelik for his unwavering support and belief in me, particularly during difficult moments in my personal life. His presence provided a much-needed source of strength and stability.

Finally, I am eternally grateful to my dear sons, Kerem and Kemal. Their presence has enriched my life beyond measure, making me a better person. Their unconditional love has been a constant source of healing and motivation throughout this endeavor.

This study was supported by the Bolu Abant Izzet Baysal University Research Foundation, Grant No: BAP 2023-TYL-6.12-0003.

1. INTRODUCTION

Over the history of humanity, plants have been crucial in both curing and preventing disease (1). The use of plants for medicinal purposes was achieved after many trial and errors. The usage of medicinal plants, local names for plants, and their techniques of use are mentioned in ancient literature, including Egyptian papyri, Hittite inscriptions, and several other sources. Plants have been used for a variety of functions, including as food, spices, colors, fuel, building materials, ornaments, magic, and superstitions (2). In the form of folk medicine and cures, Anatolia, a region where numerous civilizations have settled and numerous cultures have developed since antiquity, is home to a sizable portion of this cultural and civilizational wealth. In Turkish literature, the internationally recognized phrase "folk medicine" has the same meaning as "halk hekimliği/halk ilaçları." Folk remedies are straightforward plant-based medical concoctions that are typically based on information from ancestors and are used by rural populations to treat common diseases (3). In the Northern Iraq Region's Anidar Cave, plant samples discovered in Neanderthal tombs imply that Anatolians have been using plants as medicine since the Paleolithic epoch. These graves contain various flora including yarrow, canary grass, mallow, and sea grapes. The therapy was carried out by priest physicians in Mesopotamia beginning approximately 3000 BCE by magic and medicine, with a substantial portion of the medications being herbal treatments, according to tablets that have been preserved in the library of Nineveh. The public still uses these plant species for therapeutic reasons because of the widespread belief that they have healing capabilities. (4,5).

Throughout history, humans have been inclined to utilize various natural resources, including plants, animals, and minerals, to meet their basic needs. Findings from archaeological and ethnobotanical studies conducted to date indicate that humans have used plants to satisfy fundamental needs, such as shelter, nutrition, and healing. The word "*Ethnobotany*" comes from the Greek words "*ethno*," which means "a people who live together," and "*botany*," which means "the study of plants." John W. Harshberger, a biology professor, first discussed it in 1895 under the heading "the study of plant use by indigenous people." Ethnobotany is currently understood as the study of how plants from the surrounding environment can be used satisfy various human requirements. Several academic fields, including anthropology, archaeology, ecology, pharmaceutical botany, folklore, and art history, have been involved in ethnobotanical research (1,2).

For centuries, residents of Anatolia have employed balm and salves as part of traditional medicine, crafting concoctions from gallnut, garlic, henbane, licorice, mandrake, mint, myrtle, olives, poppy, saffron, and more to address a myriad of illnesses (4). The earliest documentation of herbal medicine in Anatolia dates back to the "De Materia Medica," scribed by the Greek physician Dioscorides in the 1st century A.D. This comprehensive study encompassed insights into approximately 950 folkloric remedies and drugs used by the Greeks and Romans (6).

An additional significant account of medicinal plant usage in Anatolia emerged during excavation works in Hattusa, the capital city of the ancient Hittite civilization. Throughout the Ottoman Empire era, herbalists served as the primary source for medicinal needs (7).

Given its strategic geographical location, Turkey possesses diverse flora and fauna. Currently, around 11.000 plant species are found in Turkey, with approximately 33% of those species being endemic. Approximately 1.000 plant species, which are found throughout the Anatolian landscape, contribute to traditional folkloric medicines (8,9). Turkey is a region where many civilizations have flourished, and populations have lived from antiquity to the present due to its different flora in each location. The compilation of knowledge from ethnobotanical studies is crucial for the preservation of indigenous plant species, cultural values, and practices (10).

1.1 Caryophyllaceae- Pink Family

The Caryophyllaceae family, recognized as a substantial and species-abundant plant family, comprises approximately 104 genera and surpasses 2630 species (11). This plant group exhibits a broad spectrum of native habitats, displaying a global presence but is absent from most wet tropics (12). Within the Caryophyllaceae family, there is a subdivision into three distinct subfamilies:

Alsinoideae (including *Areneria*, *Minuartia*, *Stelleria*, etc.), Silenoideae (encompassing *Silene*, *Dianthus*, etc.), and Paranoychioideae (comprising *Spergularia*, *Spergula*, etc.). Characterized by an herbaceous habit with both annual and perennial growth patterns, these plants have swollen nodes, a dichasial crotch of the stem, and leaves arranged in a cross-opposite manner. This family of pink-hued plants predominantly thrives in temperate and warm-temperate zones within the Northern Hemisphere, with its distribution center located in the Mediterranean region, spanning parts of Europe and Asia. The Caryophyllaceae family boasts 32 genera and approximately 500 species in Turkey (13).

1.1.1 Scientific Description of Caryophyllaceae

Herbs, both annual and perennial, are distinguished by their simple, opposite leaves that are free of stipules, or leaf-like appendages. Usually bisexual and borne singly or in clusters, the flowers have 0–5 petals and 4-5 distinct sepals, with additional internal flaps occasionally present. These plants have a variety of reproductive structures, including 3–10 stamens and a superior ovary with 1–5 chambers that can contain many or few ovules. In certain cases, the flowers are supported by a stalk called an anthophore. The fruit, which is often a capsule opening with teeth or valves that match the style number, is reached by the 2–5 styles, which can be fully or partially joined. The fruit may occasionally be a berry or exhibit irregular dehiscence. These plants are very prolific, as evidenced by the large number of seeds they produce (13).

1.1.2 Historical Application of the Caryophyllaceae Family in Traditional Medicine

Various species within the Caryophyllaceae family have found applications in traditional medicine for diverse purposes. Furthermore, certain members, notably *Gypsophila* and *Saponaria* species, hold economic significance due to their saponin content. Among these, *Saponaria officinalis* L. (soapwort) stands out as a extensively grown plant and is recognized as the most renowned species for its saponin production. The herbal properties of *Saponaria* and *Gypsophila* species are well-established, particularly in Asian traditional medicine, with China being a prominent region of authentication. Simultaneously, *Gypsophila* L. species have also been used in European folk medicine, demonstrating their usefulness across cultural boundaries (14,15).

1.2 Genera of *Dianthus*

Dianthus is a genus in the Caryophyllaceae family. This genus has over 365 species, with a worldwide distribution but centred in the Mediterranean region; some species are cultivated as ornamentals (e.g., *D. caryophyllus* L., *D. chinensis* L.) (16,17). Thirty-three of the Seventy-six *Dianthus* species that have been identified as existing in Turkey are endemic (16,18). Seven taxa (DAN, DAT, DBA, DCA, DKA, DLE, and DLY) with an endemism rate of 43.75% are endemic among the sixteen taxa in the genus *Dianthus* found in Bolu.

1.2.1 Ethnobotanical Documentation of the *Dianthus* Genus

The term "*Dianthus*" originates from two Greek words, with "*Dios*" signifying the principal deity, specifically Jupiter in Roman mythology, and "*Anthos*" translating to flower. Linnaeus bestowed this name upon the genus because of the exquisite garden flower known as the carnation (19). Renowned for its stunning flowers, the *Dianthus* genus holds particular significance in the cut flower trade, and is highly sought after for its esthetic appeal.

1.2.2 Medical Properties of different *Dianthus* Species

Table 1 presents compilation of ethnobotanical data concerning diverse *Dianthus* species. These members of the genus have found application in traditional medicine, addressing a wide spectrum of health issues, including gastrointestinal disorders, warts, common colds, coughs, hemorrhoids, tuberculosis, skin diseases, wounds, menostasis, throat infections, eye problems, diarrhea, heart diseases, toothaches, gonorrhea, headaches, stomachaches, and gum infections. For example, in traditional Chinese medicine, *Dianthus* species are used for their anti-inflammatory, analgesic, and anti-hypertensive properties. In Korean traditional medicine, it is used for its antiviral activity against herpes simplex virus. Additionally, the essential oil is commonly used as an antiseptic and as a natural remedy for toothache and sore throat (Figure 1.1).

D. anatolicus Boiss.

Common name: Yabani karanfil, Anatolian pink, Boits
Usage: Wart treatment, skin diseases, antipyretic in intermittent fever
Part of usage: Whole plant
Preperation method: General tonic
References: 20; 21; 22; 23

D. barbatus L.

Common name: Hüsnüyusuf, Sweet Williams, sakarae, willium
Usage: Stomachache, common cold, sedative, digestive, diuretic, and heart disease
Part of usage: Whole plant, seed
Preperation method: Decoction, edible
References: 24; 25; 26; 22; 23; 27

D. basuticus Burtt Davy

Common name: Drakensberg carnation, Lesotho carnation, lokoana-la-tsela / tlokofiloane
Usage: Tuberculosis, decoction for purification of blood, flatulency
Part of usage: Root
Preperation method: Fertility in bulls, root chewed as a love charm
References: 28; 22; 23; 29; 30

D. caespitosus Thunb.

Common name: Karoo pink
Usage: Chest pains, to relieve apprehension and fear
Part of usage: Whole plant
Preperation method: Love charm, used by girls initiates to wash themselves to enhance their beauty
References: 28; 29

D. carthusianorum L.

Common name: Garofano dei certosini, clusterhead pink
Usage: Antitussives, diuretics, diaphoretics, stimulants for the nervous system in cases of feebleness or nervous exhaustion
Part of usage: Whole plant
Preperation method: Infusions, syrups or the fluid extract of petals
References: 31

D. caryophyllus L.

Common name: Dağ karanfili, carnation, koronfel baladi, clavel, clavel blanco (s), cravo branco
Usage: Gum infections, gastro-intestinal disorders, wounds, throat infection, cardiotonic, emesis, diaphoretic, alexiteric, headache, asthma, dizziness, antiseptic, analgesic, skin toner and perfuming (ritual), vermifuge
Part of usage: Flowers, roots
Preperation method: As an ingredient of the "St. John's water" (cold macerate in water of a few species); Infusion, decoction
References: 32; 33; 26; 34; 35; 22; 36; 23; 37; 38

D. crinitus Sm.

Common name: Mikhak, Shafer
Usage: Toothache, stomach tonic, fever, asthma
Part of usage: Flowers
Preperation method: General tonic
References: 39

D. calocephalus Boiss.

Common name: Orman karanfili
Usage: Ornamental plant
Part of usage: Flowers
References: 40

D. chinensis L.

Common name: Çin Karanfili, China pinks, Pink, Shizhu
Usage: Cancer, menostasis, gonorrhea, coughs, diuretic, emmenagogue
Part of usage: Whole plant
References: 41; 42; 22; 23

D. deltoides L.

Common name: Bakire pembesi, Maiden pink
Usage: Stomach ache in infants, headache
References: 24

Figure 1.1. Medical properties of different *Dianthus* species

D. elegans d'Urv. var. *elegans*

Common name: Karanfil
Part of usage: Fresh roots
Preperation method: Tea; Decoction
References: 43

D. floribundus Boiss.

Common name: Nıkıla dika, Nıkla dika
Part of usage: Fresh flowers
Preperation method: Food, sucking flower nectar
References: 44

D. hymenolepis Boiss.

Common name: Nıkıla dika, Nıkla dika
Preperation method: Food, sucking flower nectar
References: 44

D. hafezi Assadi, sp. *nova.*

Common name: Mikhak Hafezi
Part of usage: Aerial parts
Preperation method: Toothache; Paste
References: 45

D. judaicus Boiss.

Common name: Clove
Usage: Dental and throat pain
Preperation method: Chewing
References: 46

D. knappii Asch. & Kanitz ex Borbás

Common name: Bosna Knapov karanfil, Knapp's carnation
Part of usage: Allergies
Part of usage: Aerial parts
Preperation method: Herbal tea
References: 47

D. micropetalus Ser.

Common name: Grassveld pink
Part of usage: Roots, whole plant
Preperation method: Steam inhalation to open the chest, boiling of root in water, addition of a burnt porcupine quill and drink; Decoction
References: 48

Dianthus mooiensis F.N Williams subsp. *kirkii* (Burtt Davys) SS Hooper

Common name: Tlhokalatsela
Usage: Genital warts
Part of usage: Roots
References: 49;50

D. orientalis Adams

Common name: Yayla karanfili, Gole Mikhak, Mikhak, Shafer
Usage: Toothache, headache, asthma, liver and kidney problems, hiccup and appetizer, for postpartum women, anti-spasm, pain reliever, calmative, mouth aroma
Part of usage: Buds, branches, flowers, fruits, aerial parts
Preperation method: Infusion, tea
References: 49; 51; 52; 39

D. plumarius L.

Common name: Clavel, clavel blanco, clavel rojo, clavilina
Part of usage: Eyes problems, cataracts, heart pain, included in mesa de challa
Usage: Ornamental plant
Part of usage: Flowers, petals
Preperation method: Infusion
References: 36

D. strictus Banks et Sol. var. *strictus*

Common name: Dimisok, Koronfel barrieh, Wild pink
Usage: Toothache, gum inflammations, liver tonic, laxative
Part of usage: Flowers, fruits
Preperation method: Decoction
References: 53; 54; 38

D. pubescens Sibth.& Sm.

Common name: Gelincik
Usage: As an ointment for the newborn babies on the head
Part of Usage: Flowers
References: 55

Figure 1.1. continued

D. superbus L.

Common name: Fringed pink, Ou mai
Usage: Cancer, heart diseases, scalp for strengthening the hair, and as washing for eyes and for skin dermatoses, asthma, carbuncles, urinary tract infections
Part of usage: Root, whole plant
Preperation method: Decoction
References: 41; 56; 23; 57; 58

D. seguieri Vill.

Common name: Clavell de muntanya
Usage: Diarrhea
Part of usage: Flowers
Preperation method: Decoction
References: 59

D. thumbergii S.S.Hooper

Common name: Thunberg's wild pink, Indlela-zimhlope
Usage: Chest pains, to relieve apprehension and fear, diabetes
Part of usage: Fresh roots
Preperation method: Oral consumption of fresh crushed roots
References: 29; 60

D. tripunctatus Sibth. & Sm

Common name: Siğil otu
Part of usage: Warts
Part of usage: Above-ground part
Preperation method: Decoction, tea
References: 61

D. zonatus Fenzl. var. *aristatus (Boiss.) Reeve*

Common name: Basur otu
Usage: Hemorrhoids
Part of usage: Flowers
Preperation method: Decoction
References: 62

D. zonatus Fenzl var. *zonatus*

Common name: Siğilotu, çingene kızı, deli karanfil
Usage: Skin diseases, tooth and gum pain, common cold, analgesic, fresh flowers are meshed and applied on the skin to remove warts
Part of usage: Flower, seed

Preperation method: Infusion, as a spice in meals, chewing the seeds.
References: 63; 64; 62; 65; 66

D. zonatus Fenzl.

Common name: Dağ karanfili, karanfil, küçük karanfil çiçeği
Usage: Warts, corns
Part of usage: Flowering branches
Preperation method: Lotion
References: 67

Dianthus spp.

Common name: Mikhak
Usage: Toothache
Part of Usage: Seed
References: 68

Figure 1.1. continued

1.2.3 Biological Activities of Genus *Dianthus*

The therapeutic potential of this genus is demonstrated by the long history of the traditional use of crude extracts from different *Dianthus* spp. for the treatment of wide range of illnesses and ailments. It is essential to understand the biological activities of these taxa in order to advance phytochemical investigations. Research on natural substances derived from various *Dianthus* spp. has produced insightful findings supported by specific references:

D. superbus has been associated with proliferative, antioxidant, cytotoxic, and antimicrobial activities (69,70,71). Similarly, *D. superbus* var. *longicalycinus* herbs has shown analgesic, antihepatotoxic, and cytotoxic effects (70,72,73).

D. coryophyllus exhibited antibacterial and antifungal activities (74,75,76). Martineti et al. (77) found that the species *D. caryophyllus* demonstrated cytotoxic activity against HCT8 human colon cancer cells in another investigation (77).

Antioxidant and antimicrobial properties were observed in *D. elegans* d'Urv. var. *elegans*, *D. erinaceus* Boiss.var. *erinaceus*, *Dly* Boiss., and *D. zonatus* (78). *D. coryophyllum* demonstrated antifungal and antibacterial activities (79), while *D. sylvestris* Wulffe displayed cytotoxic activity (80).

The hypotensive, antihelminthic, intestinal peristaltic, antitumor, and antioxidant activities of *D. chinensis* have been determined (81,82,83). The essential oil content of *D. rupicola* Biv. has been investigated. Volatile oils obtained through steam distillation were subjected to GC and GC-MS analyses. Thymol and carvacrol were identified in high proportions through the analyses. It was discovered that the extracted oils had notably poor antioxidant activity and strong antibacterial effect against *Bacillus cereus* and *B. subtilis* (84). These diverse biological activities underscore the therapeutic potential of *Dianthus* spp. and contribute valuable insights for further phytochemical research in the field (69,70,71,72,73,74,75,76,78,79,80).

1.2.4 Botanical Introduction and Distribution of studied *Dianthus* spp.

This plant can be an annual or perennial herb, sometimes woody at the base. It possesses long, thin leaves parallel to its veins. Its flowers bloom alone or in clusters, sometimes in a rounded head. Tiny bracts hug the base of the cup-shaped calyx, which lacks filmy edges or veiny seams and has five teeth. Petals on long stalks may have smooth edges, teeth, or fringes, but never split in two and lack extra flaps. Ten stamens and two styles support a capsule that opens with four teeth. Flat, shield-shaped seeds wear their scar on the front (85).

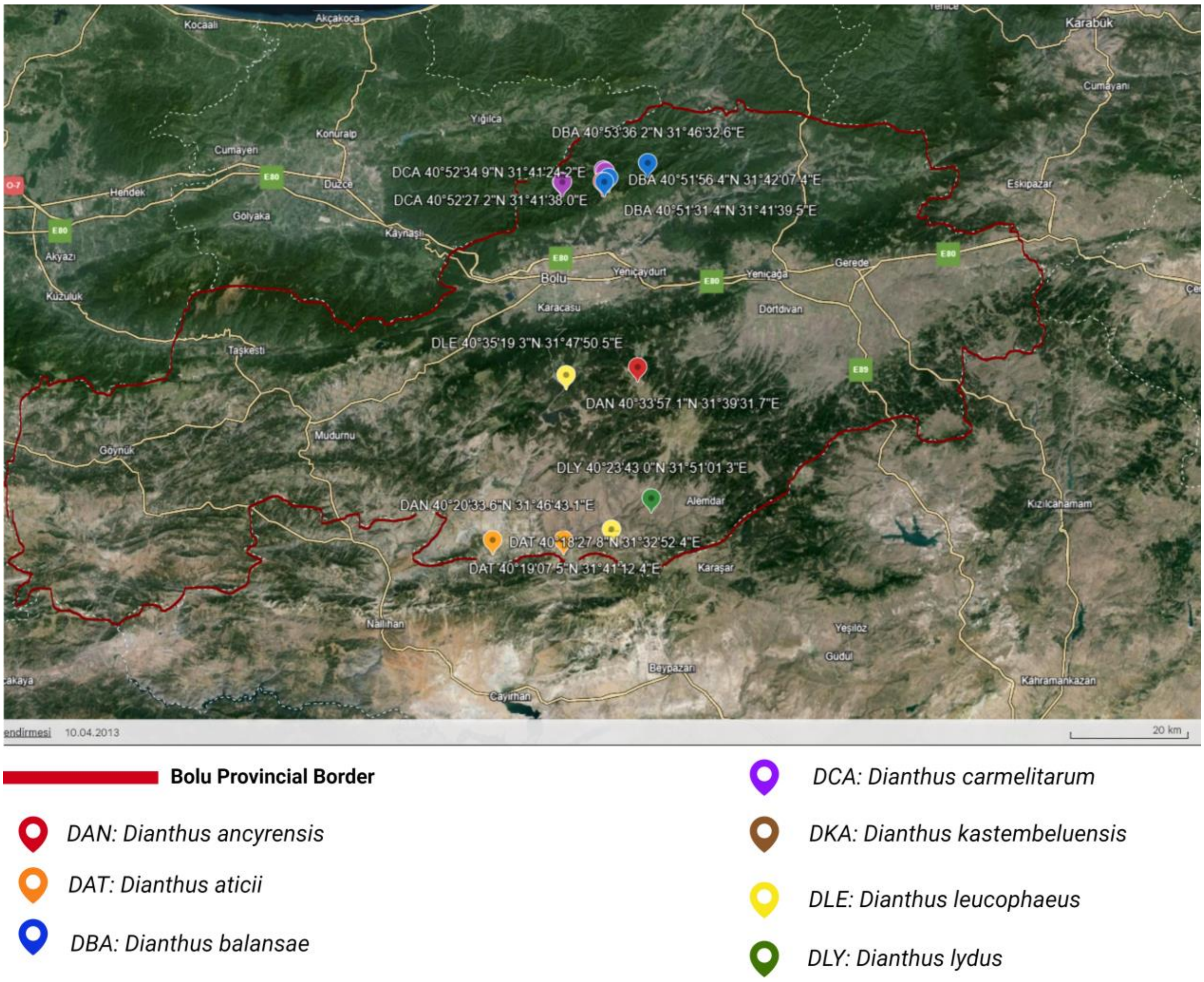


Figure 1.2. Distrubition of endemic *Dianthus* spp. in Bolu (86).

1.2.4.1 Botany and Distribution of *D. ancyrensis* Hausskn. & Bornm.

It is a perennial herb that grows to 3-15 cm with narrow leaves and small pink flowers. It blooms between June and August and is found in steppes and stony areas at an elevation of 1700-1800 meters. This species is native to Ankara and Bolu, Turkey (87).

Figure 1.3. Pictures of natural and herbarium specimen of endemic *D. ancyrensis* (DAN) (photographed A. Çelik)

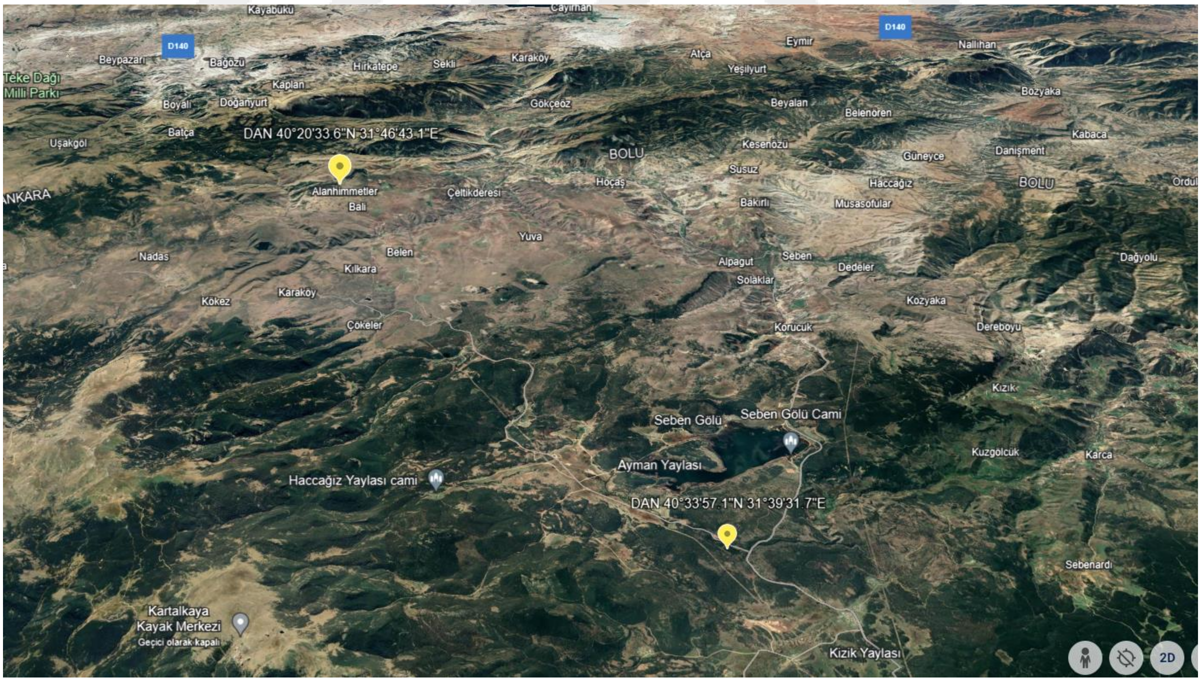


Figure 1.4. Distribution map of DAN (86).

1.2.4.2 Botany and Distribution of *D. aticii* Hamzaoğlu

It is a shrubby perennial herb that grows to 20-35 cm with thick, hairless or slightly hairy leaves and pink flowers. It blooms between June and July and is found in stony slopes and steppes at an elevation of 330-1025 meters. This species is native to Ankara, Bolu, and Bilecik (88).

Figure 1.5. Pictures of natural and herbarium specimen of endemic *D. aticii* (DAT) (photographed by A. Çelik).

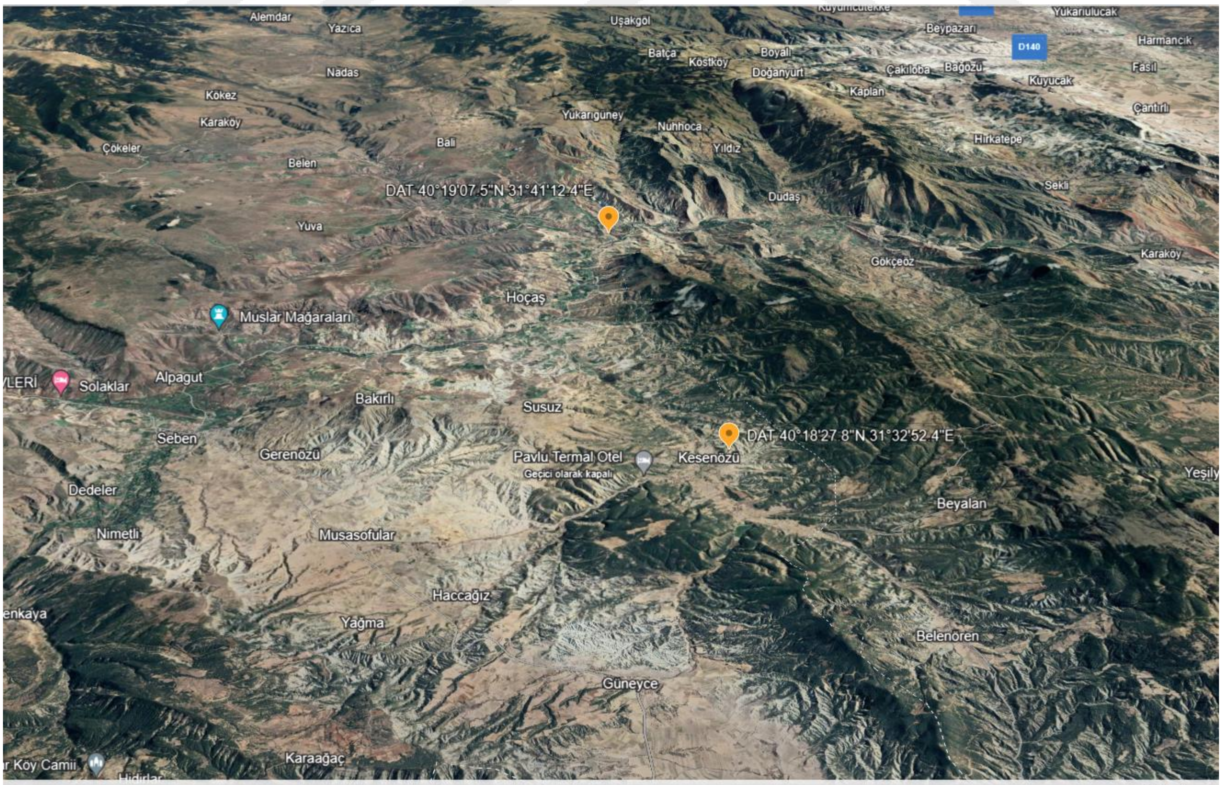


Figure 1.6. Distribution map of DAT (86).

1.2.4.3 Botany and Distribution of *D. balansae* Boiss.

It is a perennial herb that grows to 10-24 cm with long, narrow leaves and pale pink to pink flowers. It blooms between July and September and is found in rocky slopes (often limestone) at an elevation of 1800 to 2900 meters. This species is native to Ankara, Bolu, Çankırı, Erzincan, Kastamonu, Kayseri, Maraş, and Zonguldak (87).

Figure 1.7. Pictures of natural and herbarium specimen of endemic *D. balansae* (DBA) (photographed by A. Çelik).

Figure 1.8. Distribution map of DBA (86).

1.2.4.4 Botany and Distribution of *D. carmelitarum* Reut. ex Boiss.

It is a perennial herb that grows to 15-40 cm with long, narrow leaves and striking carmine red flowers. It blooms between June and August and is found in banks and field edges at elevation of 300 to 3000 meters. This species is native to Bolu, Erzurum, Giresun, Gümüşhane, Ordu, Rize, Sinop, and Trabzon (87).

Figure 1.9. Pictures of natural and herbarium specimen of endemic *D. carmelitarum* (DCA) (photographed by A. Çelik).

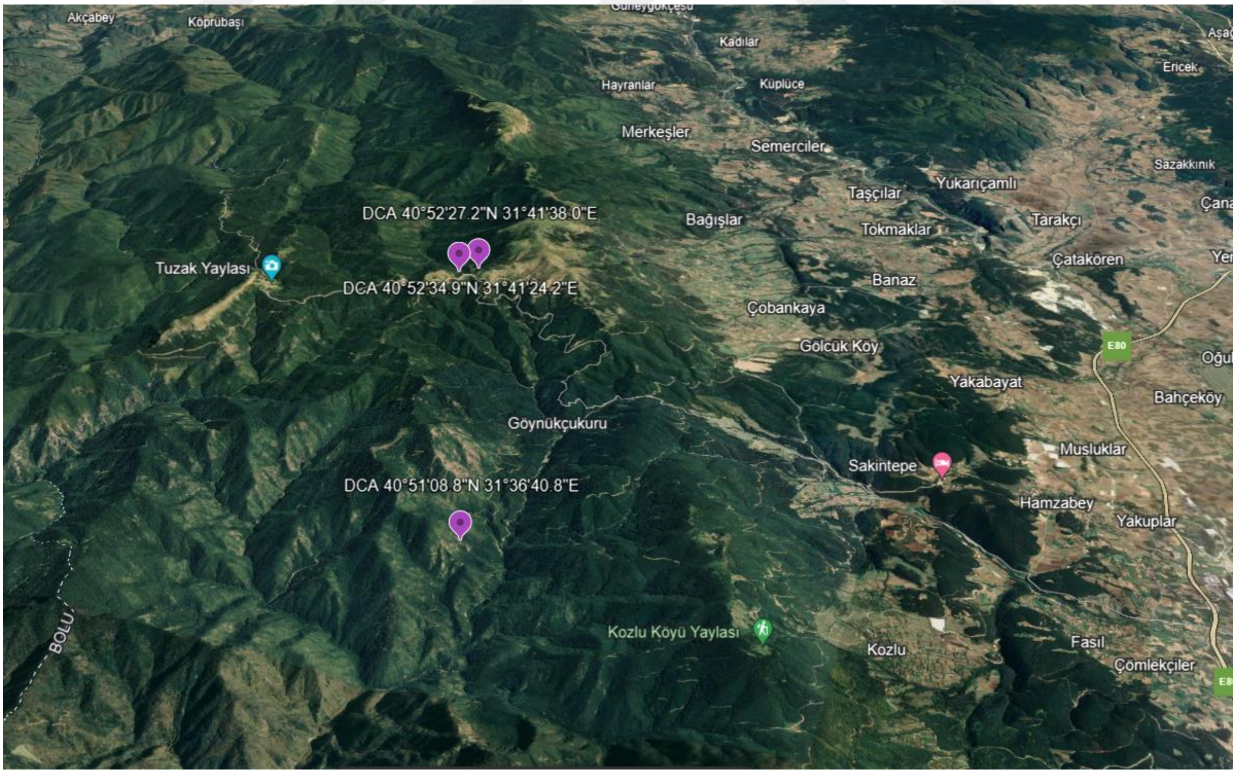


Figure 1.10. Distribution map of DCA (86).

1.2.4.5 Botany and Distribution of *D. kastembeluensis* Freyn & Sint.

It is a hairy perennial herb that grows to 5-40 cm with a woody base, narrow leaves, and charming pink flowers. Its blooms in August and is found in a touch of color to cliffs at elevation of 50 to 1300 meters. This species is native to Bolu, Kastamonu, and Ankara (87).

Figure 1.11. Pictures of natural and herbarium specimen of endemic *D. kastembeluensis* (DKA) (photographed by A. Çelik).

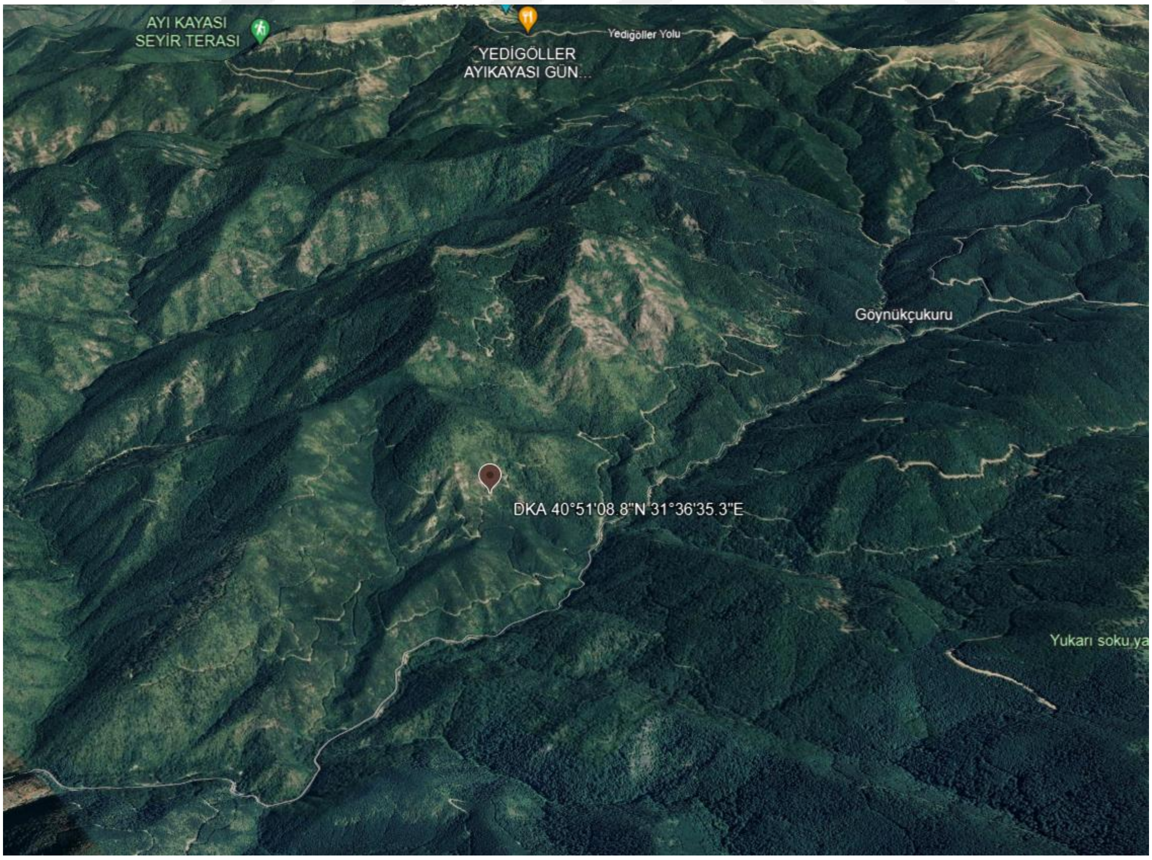


Figure 1.12. Distribution map of DKA (86).

1.2.4.6 Botany and Distribution of *Dianthus leucophaeus* Sm.

It is a perennial herb that grows to 18-45 cm with slender leaves and delicate white to pinkish flowers create a charming appearance. It blooms between June and September and is found in mountainous areas with its presence at elevation of 1500 to 2400 meters. This species is native to Bolu, Burdur, Bursa, Isparta, İzmir, Konya, Ordu, and Sinop (87).

Figure 1.13. Pictures of natural and herbarium specimen of endemic *D. leucophaeus* (DLE) (photographed by A. Çelik).

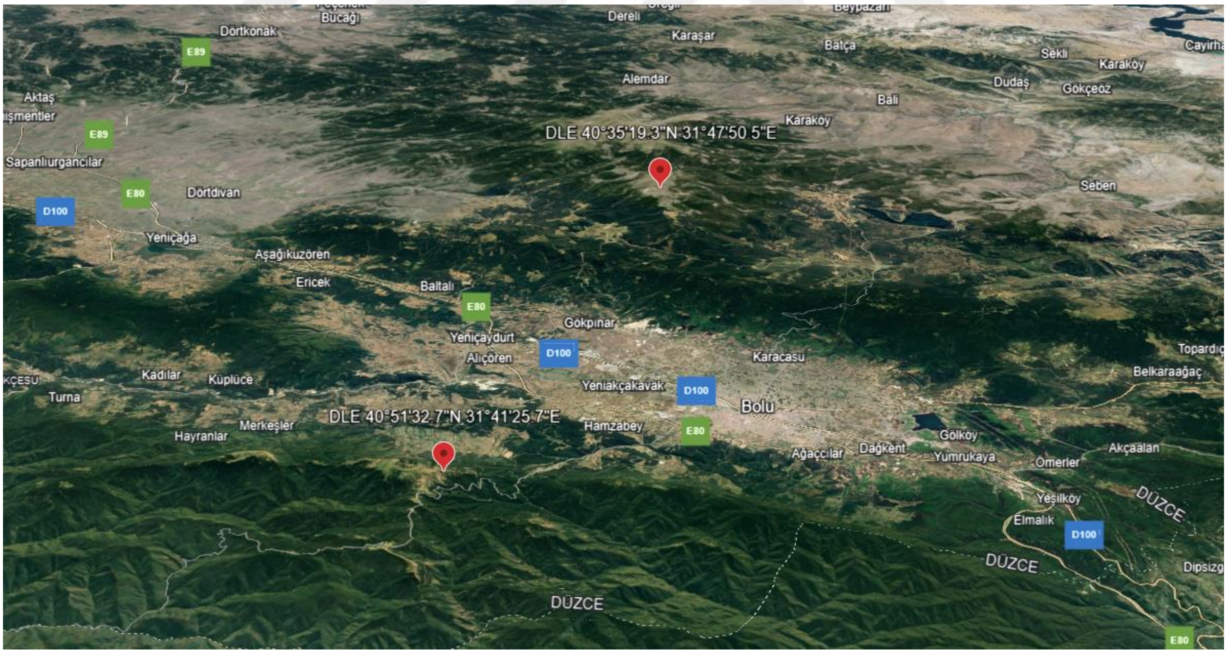


Figure 1.14. Distribution map of DLE (86).

1.2.4.7 Botany and Distribution of *Dianthus lydus* Boiss.

It is a tall perennial herb that grows to 25-60 cm with long, thin leaves alongside striking red flowers. It blooms between June and July and is found in a touch of color to rocky areas. This species is native to Bolu, Izmir, Çanakkale, Manisa, and Ankara (87).

Figure 1.15. Pictures of natural and herbarium specimen of endemic *D. leucophaeus* (DLY) (photographed by A. Çelik).

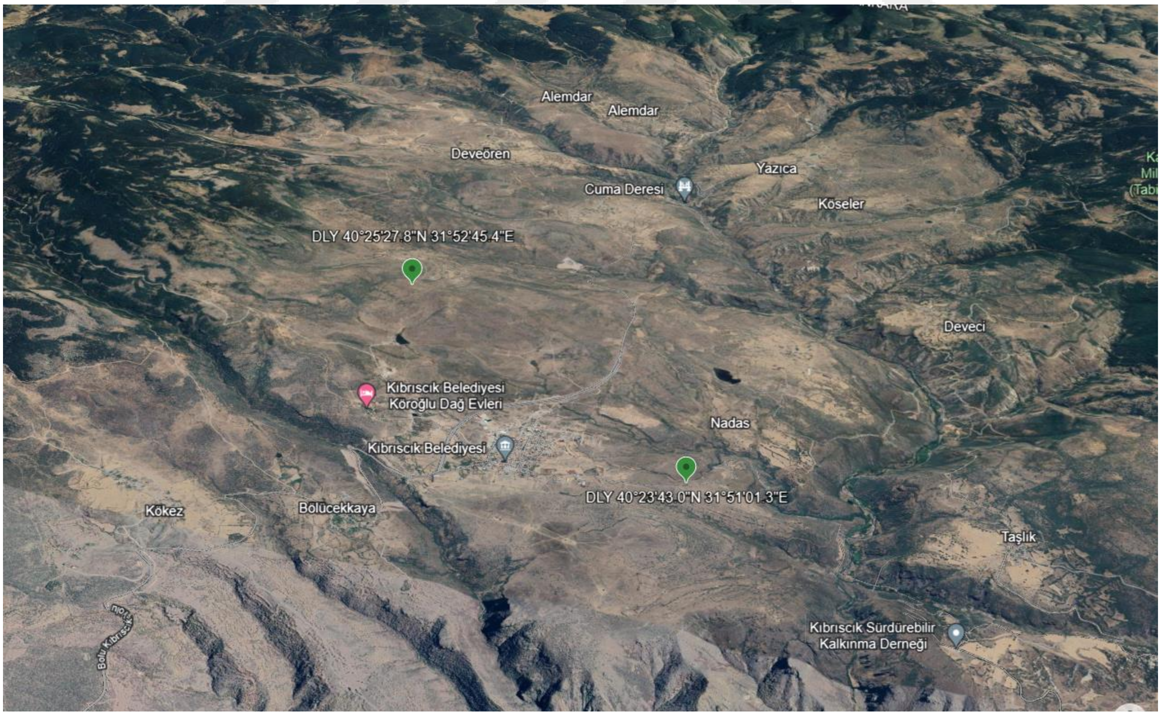


Figure 1.16. Distribution map of DLY (86).

2. AIM AND SCOPE OF THE STUDY

- Identification of endemic *Dianthus* spp. found in Bolu.
- Collection of seven Bolu-based endemic *Dianthus* spp. (*D. aticii, D. ancyrensis*, *D. carmelitarum*, *D. balansae*, *D. lydus*, *D. leucocephalus*, and *D. kastemboluensis*) from their natural habitats.
- Desiccation of the collected plants on drying racks avoiding the sun, separation of each plant species into 2 different parts (FC - flower+capitulum and LS - leaf+stem), pulverization of the parts and preparation of methanol extracts from fourteen different samples.
- Determination of antioxidant and antibacterial potentials of methanolic extracts of fourteen plant extracts.
- Measurement of total phenol and flavonoid content in fourteen distint plant extracts.
- Characterization and quantification of individual phenolic constituents found in fourteen plant extracts with UHPLC.

3. MATERIALS AND METHODS

3.1 Plant Material and Extraction Method

Seven endemic *Dianthus* spp., including DAN, DAT, DBA, DCA, DKA, DLY, and DLE were collected from Bolu, voucher specimens were deposited at Bolu Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. Detailed information, including the distribution, common names, and collection numbers, for each plant sample was demonstrated in Table 3.1.

To extract the plant materials, two distinct components [(FC - flower+capitulum (1) and LS - leaf+stem (2)] were utilized. Dried and powdered plants, 10 grams of plant materials were subjected to extraction with methanol (MeOH) at 40 ºC. After 24 hours, methanolic extracts were then concentrated by rotary evaporator. The extraction yields were presented in Table 3. 1.

Table 3. 1. Designation of studied plant extracts, botanical and Turkish names, used parts, collection numbers, distribution, and extraction yield (%) of the plants.

Endemic *Dianthus* spp.	Used parts	Collection number	Turkish names	Distribution in Turkey	Extraction yield (%)
Dianthus ancyrensis Hausskn. & Bornm. .	FC	AYS1530	Ankara karanfili	Ankara and Bolu	11.78
	LS				11.62
Dianthus aticii Hamzaoğlu	FC	AYS1515	Atici karanfili	Ankara, Bolu and Bilecik	7.14
	LS				7.34
Dianthus balansae Boiss.	FC	AYS1504	Aslan karanfli	Bolu, Ankara, Kastamonu, Çankırı, Zonguldak, Maraş, Kayseri and Erzincan	10.42
	LS				10.17
Dianthus carmelitarum Reut. ex Boiss.	FC	AYS1503	Samsu	Bolu, Sinop, Ordu, Gireysun, Gümüşhane, Erzurum, Rize and Trabzon	4.8
	LS				6.51
Dianthus kastembeluensis Freyn & Sint.	FC	AYS1500	Kastamonu karanfili	Bolu, Kastamonu, and Ankara	14.02
	LS				10.73
Dianthus leucophaeus Sm.	FC	AYS1521	Hoş karanfil	Bolu, Sinop, Ordu, Bursa, Bolu, İzmir, Burdur, Isparta and Konya	4.64
	LS				6.81
Dianthus lydus Boiss.	FC	AYS1532	Kan karanfil	Bolu, İzmir, Çanakkale, Manisa and Ankara	7.58
	LS				8.94

3.2 Determination of Antioxidant Properties

3.2.1 DPPH Assay (2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity Assay)

The Blois method (89) was used for the assessment (90,91). The free radical scavenging capacity of *Dianthus* spp. was identified by spectrophotometric means using the DPPH radical analysis. The absorption of each sample at 517 nm was measured against a blank using a UV-VIS spectrophotometer (Hitachi U1900®). All analyses were conducted in triplicate to ensure the reliability and consistency of the obtained results.

3.2.2 Total Phenolic Content (TPC) by Folin-Ciocalteu Assay

Total phenolic content (TPC) of the extracts was determined using Folin-Ciocalteu assay (91).

3.2.3 Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) of the extracts was determined using aluminum colorimetric test (91).

3.3 Antibacterial Bioassay

The disc diffusion assay (Kirby-Bauer Method) was used to evaluate for antibiotic activity (91,92). Human and fish pathogens were tested with help of the disc diffusion technique (93) (Table 3.2).

Table 3. 2. Human and fish pathogens used in the experiment.

Human Gram Positive Pathogens		**Fish Gram Positive Pathogens**
Streptococcus pyogenes	ATCC© 19615	*Enterococcus faecalis*
Staphylococcus aureus	ATCC© 25923	*Lactococcus garvieae*
Staphylococcus epidermidis	ATCC© 12228	*Streptococcus agalactiae*
Human Gram Negative Pathogens		**Fish Gram Negative Pathogens**
Escherichia coli	ATCC© 25922	*Aeromonas hydrophila*
Pseudomonas aeruginosa	ATCC© 27853	*Aeromonas salmonicida*
Salmonella typhimurium	ATCC© 14028	*Vibrio anguillarum*
Serratia marcescens	ATCC© 8100	*Yersinia ruckeri*
Proteus vulgaris	ATCC© 13315	
Enterobacter cloacae	ATCC© 23355	
Klebsiella pneumoniae	ATCC© 13883	

3.4 Ultra High Performance Liquid Chromatography (UHPLC) Analysis

The analysis through Ultra-High Performance Liquid Chromatography (UHPLC) was conducted at BAIBU Central Laboratory (BETUM) according to Celik et al. (94) (Figure 3.1.).

3.5 Data Analysis

Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests were conducted using SPSS version 26 (IBM Corp, NY, USA). Results presented in the tables were expressed as the mean number ± standard error (SE). Pearson's correlation analysis was used for the indication of the relationship.

4. RESULTS AND DISCUSSION

4.1 Antioxidant Capacity

Antioxidant molecules are essential for preventing or delaying the oxidation process. Reactive oxygen species (ROS) are inevitable byproducts of cellular aerobic metabolism (95). The accumulation of ROS can cause oxidative stress, a condition that, if not controlled, may contribute to various pathological states (96). Antioxidants, a diverse group of molecules, can disrupt radical reactions, thereby preventing cellular damage (97).

The *Dianthus* spp. that were the subject of the study showed different levels of antioxidant activity. Table 4.1 shows that DBA2 and DAT2 had the highest capacity to scavenge free radicals. Interestingly, the DAN2, DAT, DBA, DCA, and DKA have demonstrated stronger antioxidant capacity than FC parts, with the exception of DLY and DLE. According to the antioxidant results, DAT and DBA2 had the highest TFC and TPC, respectively (Table 4.1). The antioxidant activity of the methanolic extracts of LS and FC was found to be lower in our study compared to the results of Aliyazicioglu et al. (98) on the antioxidant activity of the aqueous extract of the aerial part of DCA (1.23 mg/ml) (Table 4.1.). However, compared to the previous study's value of 12.6 mg GAE/g (98), our study reported higher TPC in DCA (34.99 and 20.70 mg GAE/g, respectively). Similarly, in the DMSO extract taken from the aerial part of DCA, Turan et al. (99) found that TPC and TFC were 7.85 mg GAE/g and 6.36 mg QE/g, respectively. Although TPC and TFC as wells as antioxidant capacity of DAN, DBA, DAT, DKA, DLY, and DLE had not been investigated before, several studies have investigated distinct *Dianthus* spp. For instance, Cai et al. (100) discovered that the aerial portion of *D. superbus*'s methanolic extract had a TPC of 5.9 mg GAE/g. According to Gou et al. (71) the TPC and TFC of *D. suberbus*-LS was 3.27 mg Rutin E/g and 24.04 mg GAE/g, respectively, with an IC_{50} value of 0.9 mg/ml for the aqueous extract. The aqueous, ethanolic, and hydroethanolic extracts of the entire *D. basuticus* plant were found to have IC_{50} values of 2.56 µg/mL, 66.78 µg/mL, and 44.10 µg/mL, respectively, by Kazeem et al. (101).

Table 4. 1. Free radical scavenging activity, total phenolics and flavonoids capacity (TPC and TFC) of tested *Dianthus* spp. IC_{50}: The half maximal inhibitory concentration. GA: Gallic acid, Q:

***Dianthus* spp.**	**Parts**	**IC_{50} DPPH Inhibition (mg/ml)**	**Total Phenol (mg GAE/g dry sample)**	**Total Flavonoid (mg QE/g dry sample)**
D. ancyrensis	FC	5.14±0.18^{h}	28.41±2.97^{e}	66.37±1.31^{d}
	LS	2.23±0.00^{c}	33.94±2.61^{cd}	59.77±0.33^{e}
D. aticii	FC	4.68±0.02^{g}	36.33±1.29^{c}	60.97±2.06^{e}
	LS	2.06±0.09^{c}	62.00±1.56^{a}	86.46±1.74^{a}
D. balansae	FC	2.79±0.17^{d}	45.96±1.52^{b}	59.89±1.02^{e}
	LS	1.22±0.03^{b}	65.89±1.56^{a}	69.42±2.05^{d}
D. carmelitarum	FC	7.07±0.23^{i}	20.70±0.66^{f}	24.62±0.64^{i}
	LS	3.72±0.20^{e}	34.99±1.44^{cd}	53.89±1.37^{f}
D. kastembeluensis	FC	8.18±0.06^{j}	26.35±1.59^{e}	80.29±2.18^{b}
	LS	3.96±0.04^{ef}	33.67±1.22^{cd}	75.50±0.85^{c}
D. lydus	FC	3.76±0.06^{e}	38.11±0.44^{c}	39.83±0.67^{h}
	LS	4.82±0.13^{gh}	28.39±0.72^{e}	45.17±2.09^{g}
D. leucophaeus	FC	3.89±0.17^{ef}	44.78±0.11^{b}	51.53±1.05^{f}
	LS	4.14±0.05^{f}	30.38±1.45^{de}	54.73±0.75^{f}
Quercetin		0.04±0.00^{a}		

Free radicals are scavenged and inhibited in large part by the antioxidant compounds present in the plant materials. The observed radical scavenging activity of the tested *Dianthus* spp. and the TPC were found to significantly correlate ($r = -0.79$, $P < 0.05$).

Although the results of our study on antioxidant activity are in line with previous research, discrepancies could potentially result from differences in plant species, the existence of phenolic substances in plants that serve particular purposes, the kind of extraction method employed, geographic conditions, harvesting seasons, and post-harvest conditions.

4.2 Antibacterial Activity

Among the 10 tested human pathogens, only *S. aureus*, *S. epidermidis*, *S. pyogenes*, and *K. pneumonia* exhibited sensitivity to some investigated *Dianthus* spp. Moderate susceptibility was observed with *S. epidermidis* to DLE2 and with *S. pyogenes* to DLY1, DAT1, and DKA (Table 4.2). However, none of the tested fish pathogens showed susceptibility to any extract of *Dianthus* spp. (Table 4.3). Reference antibiotics, used as positive controls, generally demonstrated antibacterial activity against the tested microorganisms. The final concentrations of all extracts were tested in methanol as a negative control, which exhibited no inhibition.

Based on ethnobotanical records of *Dianthus* spp. (Figure 1.1-continued), these plants have been traditionally used in the treatment of gum infections, sore throat, wounds, and skin diseases. The noteworthy inhibitory activity of DLE against *S. epidermidis* may explain the traditional use of some *Dianthus* spp. in skin diseases, gum infections, and wounds. Moreover, the sensitivity of *S. pyogenes* to DAT, DKA, and DLY may support the folkloric usage of these *Dianthus* spp. as remedies for sore throat, gum infections, skin diseases, and wounds.

Aliyazicioglu et al. (98) found that the aerial part of the aqueous extract of DCA had antibacterial activity against *P. aeruginosa* and *S. aureus* while having no inhibition against *E. coli* and *E. faecalis*. Conversely, DCA methanolic extracts in our investigation showed no antibacterial activity against these bacteria (Table 4.2).

Similar to our results (Table 4.2), the methanol extract of DBA did not show antibacterial effects against *S. pyogenes*, *S. aureus*, *P. aeruginosa*, *E. coli*, and *E. faecalis*, as reported by Benli et al. (102). The essential oils obtained from DCA did not demonstrate antimicrobial activity against various bacteria except *Candida albicans* (89).

Similar findings about the antibacterial activity of tested *Dianthus* spp. have been reported in a variety of studies. For example, Hürkül and Kıymacı (103) reported that DAN did not possess any antibacterial activity against *S. aureus*, methicillin-resistant *S. aureus*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. While the apolar extract (hexane) of DLY exhibited antibacterial efficacy against *S. epidermidis*, *E. coli*, and *P. aeruginosa*, no activity against *S. aureus* was observed (78). However, the polar extract (methanol) of DLY in our study did not exhibit activity against these bacteria, showing only inhibition against *S. pyogenes* (Table 6). The antimicrobial properties of apolar extracts might be attributed to the rich sources of unsaturated fatty acids (78). Various *Dianthus* spp. have been investigated for their antimicrobial activities. For example, the ethanolic extract of *D. caryophyllum* demonstrated moderate antibacterial activity against *E. coli*, *B. subtilis*, *S. aureus*, *S. epidermidis*, and *P. aeruginosa*, and it also demonstrated antifungal activity against *Aspergillus niger* and *C. albicans*. (79).

Furthermore, the aqueous extract of *D. basuticus* exhibited mild antibacterial action against *B. pumilis*, *E. coli*, and *S. aureus* in addition to antifungal activity against *C. rugosa*, *C. albicans*, and *Trichosporon mucoides*, according to Lamula and Ashafa (104). According to Dahiya (105) *D. superbus* exhibited modest anthelmintic action against a variety of worms and possible antibacterial activity against *C. albicans*. (105).

Table 4. 2. Antibacterial potential of tested *Dianthus* spp.

		Mean Diameter of Inhibitory Zones (mm ± SE)									
***Dianthus* spp.**	**Parts**	***S.auerus***	***S. epidermidis***	***S.pyogenes***	***S. marcescens***	***S.typhimurium***	***P.aeruginosa***	***P. vulgaris***	***K. pneumonia***	***E. cloacae***	***E.coli***
D. ancyrensis	FC	-	-	-	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-	-	-	-
D. aticii	FC	-	-	12.00 ± 0.00^{fg}	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-	-	-	-
D. balansae	FC	-	-	-	-	-	-	-	7.33 ± 0.33^{cd}	-	-
	LS	-	-	-	-	-	-	-	6.50 ± 0.50^{d}	-	-
D. carmelitarum	FC	-	-	-	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-	-	-	-
D. kastembeluensis	FC	-	-	9.67 ± 0.33^{h}	-	-	-	-	-	-	-
	LS	-	-	12.83 ± 0.17^{f}	-	-	-	-	-	-	-
D. lydus	FC	-	-	11.83 ± 0.44^{g}	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-	-	-	-
D. leucophaeus	FC	6.5 ± 0.00^{e}	12.50 ± 0.50^{c}	-	-	-	-	-	-	-	-
	LS	6.75 ± 0.25^{e}	-	-	-	-	-	-	-	-	-
Chloramphenicol		33.75 ± 0.25^{c}	34.95 ± 0.05^{a}	38.75 ± 0.25^{e}	26.75 ± 0.25^{a}	27.50 ± 0.50^{a}	-	26.75 ± 0.25^{c}	29.75 ± 0.25^{a}	31.10 ± 0.10^{a}	29.75 ± 0.25^{a}
Carbenicillin		35.75 ± 0.25^{b}	27.25 ± 0.25^{b}	51.00 ± 0.00^{b}	15.00 ± 1.00^{c}	25.25 ± 0.25^{c}	11.75 ± 0.25	33.75 ± 0.25^{a}	-	32.00 ± 1.00^{a}	23.75 ± 0.25^{c}
Erythromycin		32.5 ± 0.00^{d}	34.75 ± 0.25^{a}	47.25 ± 0.25^{d}	9.25 ± 0.25^{d}	8.75 ± 0.25^{d}	-	11.75 ± 0.25^{e}	8.25 ± 0.25^{c}	-	10.75 ± 0.25^{e}
Tetracycline		36.75 ± 0.25^{a}	10.75 ± 1.75^{c}	49.25 ± 0.25^{c}	21.25 ± 0.25^{b}	26.25 ± 0.25^{bc}	18.75 ± 0.25	32.25 ± 0.25^{b}	27.75 ± 0.25^{b}	31.75 ± 0.25^{a}	28.75 ± 0.25^{b}
Ampicillin		35.75 ± 0.25^{b}	24.95 ± 0.05^{b}	52.50 ± 0.50^{a}	-	26.75 ± 0.25^{ab}	-	22.50 ± 0.50^{d}	7.25 ± 0.25^{cd}	19.25 ± 0.25^{b}	19.25 ± 0.25^{d}
Methanol		-	-	-	-	-	-	-	-	-	-

Table 4. 3. Antibacterial potential of tested *Dianthus* spp.

		Mean Diameter of Inhibitory Zones (mm ± SE)						
***Dianthus* spp.**	**Parts**	***S. agalactiae***	***L. garviaeae***	***E. faecalis***	***A. hydrophila***	***V. anguillarum***	***Y. ruckeri***	***A. salmonicida***
D. ancyrensis	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. aticii	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. balansae	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. carmelitarun	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. kastembeluei	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. lydus	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
D. leucophaeus	FC	-	-	-	-	-	-	-
	LS	-	-	-	-	-	-	-
Furazolidone		8.95 ± 0.05^{d}	12.25 ± 0.25^{b}	15.25 ± 0.25^{b}	31.25 ± 0.25^{d}	16.25 ± 0.25^{b}	12.75 ± 0.25^{c}	31.00 ± 1.00^{a}
Sulphamethoxazole		21.75 ± 0.25^{c}	-	-	38.25 ± 0.25^{b}	-	31.75 ± 0.25^{a}	16.50 ± 0.50^{b}
Erythromycin		32.75 ± 0.25^{a}	26.25 ± 0.25^{a}	18.75 ± 0.25^{a}	34.25 ± 0.25^{c}	18.75 ± 0.25^{a}	8.75 ± 0.25^{d}	8.50 ± 0.50^{c}
Tetracycline		31.75 ± 0.25^{b}	25.25 ± 0.25^{a}	15.75 ± 0.25^{b}	41.75 ± 0.25^{a}	15.50 ± 0.50^{b}	27.75 ± 0.25^{b}	28.50 ± 0.50^{a}
Methanol		-	-	-	-	-	-	-

Figure 4. 1. Antibacterial effect of *D. leucophaeus*-FC and LS (13 and 14) against *S. aureus* (Sa).

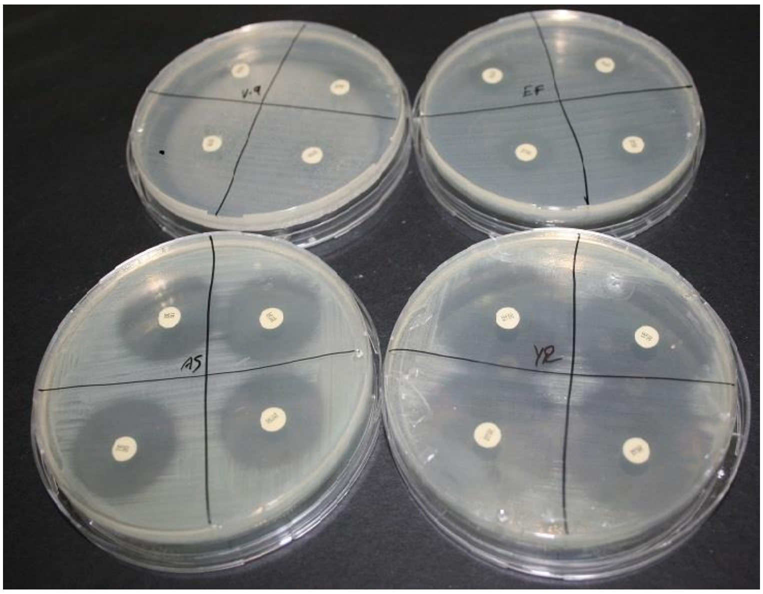


Figure 4. 2. Antibacterial effects of positive control (antibiotic-tetracycline) against fish pathogens [*V. angulliarum* (Va), *E. faecalis* (Ef), *A. salmonicida* (As) and *Y. ruckeri* (Yr)].

4.3 UHPLC Analysis

The characterization of twelve phenolics was identified, and the chromatogram of the used standards was indicated in Figure 4.3 The UHPLC study revealed that rutin was the most dominant component in *Dianthus* spp. as a result of the total quantity of selected phenolics (QSP). In particular, all tested parts (LS and FC) of DCA were determined to be the best rutin sources (Table 4.4). DLY1 and DAN1 also had higher amounts of rutin. The highest overall QSP was determined in the aerial parts of DCA and DAN. The high antioxidant capacity of DCA may be associated with the high content of rutin, which is a type of flavonoid that naturally occurs in particular plants. It possesses anti-inflammatory and antioxidant properties, which can help protect the health of blood vessels. Because of these properties, this compound can strengthen capillaries and provide protection against circulation disorders. Besides, it might strengthen the immune system, improve skin health and, diminish allergy symptoms (106). Phloridzin was identified as the second dominant compound due to overall QSP. DAN2 had the highest phloridzin content, followed by DCA1 and DAN1. The strong antioxidant characteristics of DAN and DCA may be related to their phloridzin content together with rutin. Phloridzin, also known as phlorizoside or phlorrhizin, is a chemical compound classified as a flavonoid O-glycosides. Several investigations have shown that phloridzin possesses a wide range of biological activities, such as antioxidant, anti-diabetic, anti-hyperglycemic, antibacterial, anti-obesity, anti-inflammatory, cardioprotective, anti-cancer, neuroprotective, hepatoprotective, and immunomodulatory activities (107). Because of its antioxidant capacity, chlorogenic acid is capable of decreasing oxidative stress in cells, hindering the negative effects of free radicals. It helps to regulate blood sugar and is considered to provide a variety of health benefits, including improved heart health, weight loss, and protection against Alzheimer's disease and cancer (108). Among the species to be researched, individual phenolic analyses of DAT, DKA, DLY, and DLE have not been investigated previously. Sahin (90) identified that the methanolic extract of DAN aerial part had gallic acid, chicoric acid, 4-hydroxybenzoic acid, ferulic acid, chlorogenic acid, gentisic acid, vanillic acid, p-coumaric acid, quercetin, and rutin.

In addition to the methanolic DBA extract, the following phenolic quantities were found in decreasing order: rutin, vanillic acid, chicoric acid, 4-hydroxybenzoic acid, gentisic acid, ferulic acid, p-coumaric acid, gallic acid, and quercetin. Consistently in the present study, the phenolic content of DAN followed the order of decreasing amounts as phloridzin, rutin, o-coumaric acid, ferrulic acid, chlorogenic acid, gallic acid, caffeic acid, vanillic acid, and protocatecuic acid. Moreover, the following compounds were present in decreasing order in DBA: rutin, caffeic acid, ferrulic acid, phloridzin, vanillic acid, syringic acid, ocoumaric acid, and p-coumaric acid. Aliyazicioglu et al. (92) identified chlorogenic acid and gallic acid in the aqueous extract of the aerial portion of DCA; however, these two phenolic acids were absent in the methanol extract of DCA in the present investigation (Table 7). Syringic acid, protocatecuic acid, p-coumaric acid, caffeic acid, and vanillic acid were the most common phenolic components detected in DCA extracts in both studies (Table 7) (93). Turan et al. (91) identified benzoic acid, syringaldehyde, p-coumaric acid, sinapic acid, p-OH benzoic acid, quercetin, and vanillic acid in the order of decreasing phenolic amount in the DMSO extract of DCA aerial part.

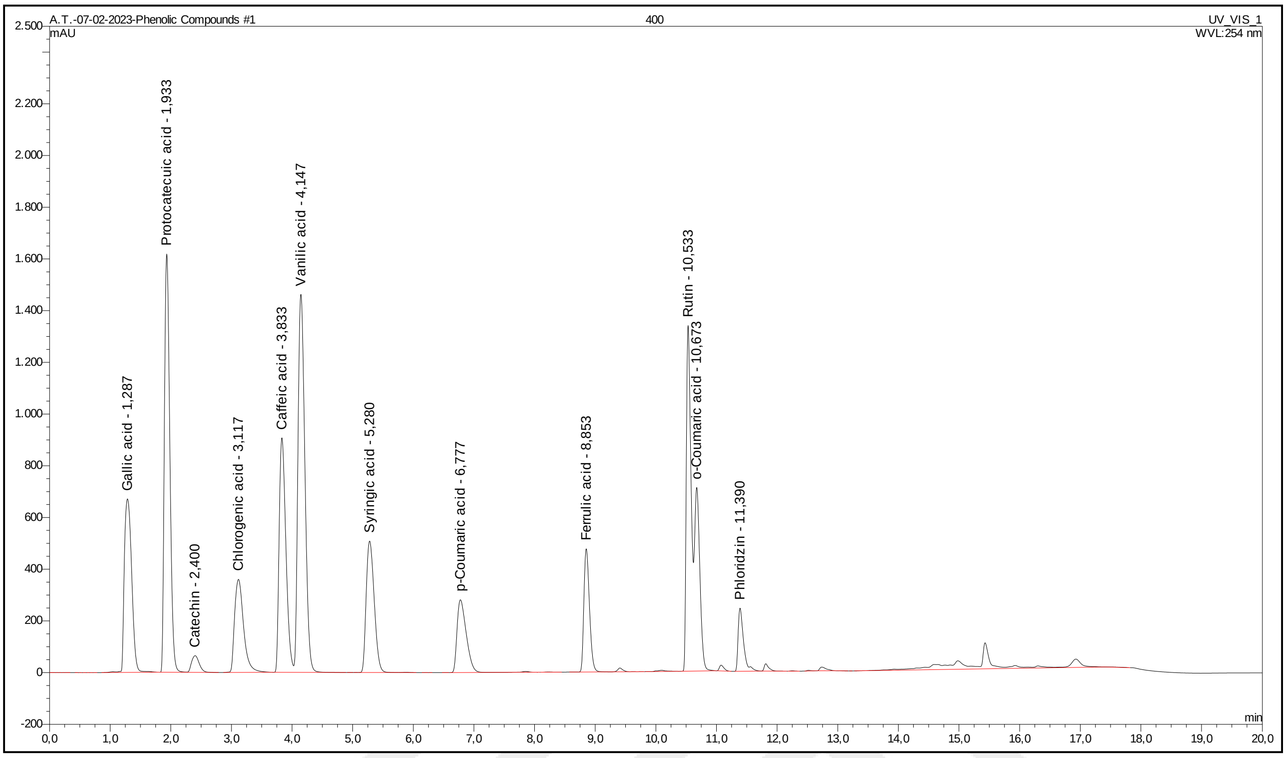


Figure 4. 3. HPLC chromatogram of 12 phenolic standards and their retention times.

Table 4. 4. Quantitative analysis of tested plant extracts by UHPLC.

		Phenolic compounds (µg/g dry weight)												
***Dianthus* spp.**	**Parts**	**Caffeic acid**	**Catechin**	**Chlorogenic acid**	**Ferrulic acid**	**Gallic acid**	**o-Coumaric acid**	**p-Coumaric acid**	**Phloridzin**	**Protocatecuic acid**	**Rutin**	**Syringic acid**	**Vanilic acid**	**Overall QSP**
D. ancyrensis	FC	40.29±8.13^{d}	-	1.88±0.01^{c}	144.71±0.40^{b}	65.45±3.33^{a}	346.89±0.93^{a}	-	209.27±1.22^{c}	3.39±0.15^{a}	304.68±4.34^{d}	-	8.71±0.64^{c}	**1116.56**
	LS	-	-	187.12±47.88^{b}	68.21±0.27^{e}	66.24±16.35^{a}	-	-	512.03±2.69^{a}	1.25±0.15^{c}	128.08±0.07^{e}	-	0.45±0.15^{c}	**962.93**
D. aticii	FC	5.41±0.27^{f}	-	-	73.46±0.21^{de}	11.41±0.28^{b}	32.29±1.16^{b}	-	9.36±0.25^{hi}	-	131.83±6.30^{e}	-	197.48±11.99^{a}	**263.76**
	LS	-	-	-	18.26±0.38^{fg}	-	32.28±0.26^{b}	-	-	-	14.69±0.39^{g}	-	1.96±0.01^{c}	**65.23**
D. balansae	FC	81.08±0.75^{b}	-	-	109.92±6.02^{c}	-	9.18±0.05^{d}	10.35±0.21^{c}	29.90±0.28^{g}	-	48.22±0.27^{fg}	-	24.01±0.54^{b}	**288.65**
	LS	112.58±5.36^{a}	-	32.18±0.46^{c}	82.88±4.30^{de}	-	12.21±0.50^{c}	-	-	-	30.74±0.26^{g}	23.07±7.61^{a}	1.17±0.04^{c}	**293.66**
D. carmelitarum	FC	111.48±0.74^{a}	-	-	86.39±15.11^{d}	-	-	11.02±0.38^{c}	230.57±4.46^{b}	-	598.86±32.17^{b}	-	1.33±0.04^{c}	**1038.32**
	LS	74.64±0.03^{b}	-	-	-	-	-	82.08±0.00^{a}	47.62±1.24^{f}	1.30±0.00^{c}	864.72±6.07^{a}	19.19±0.49^{ab}	1.97±0.01^{c}	**1089.55**
D. kastembeluensis	FC	-	6.60±0.28	350.1±88.28^{a}	7.17±0.30^{gh}	-	7.5±1.69^{d}	6.83±0.08^{cd}	104.56±1.31^{d}	0.17±0.03^{f}	27.24±3.12^{g}	-	1.28±0.01^{c}	**510.17**
	LS	-	-	12.78±0.36^{c}	32.03±0.67^{f}	-	3.12±0.07^{e}	8.12±0.26^{cd}	8.54±0.01^{hi}	-	67.05±1.45^{f}	0.47±0.01^{c}	0.33±0.00^{c}	**132.11**
D. lydus	FC	57.03±0.73^{c}	-	22.08±2.51^{c}	163.76±10.83^{a}	-	-	4.04±0.89^{de}	65.80±14.16^{e}	1.64±0.04^{b}	356.98±24.74^{c}	16.30±1.55^{ab}	33.52±0.42^{b}	**687.63**
	LS	23.32±0.13^{e}	-	-	9.84±1.15^{gh}	-	1.10±0.00^{f}	-	19.35±0.38^{gh}	0.32±0.00^{e}	14.47±0.30^{g}	1.06±0.04^{c}	5.59±0.16^{c}	**69.46**
D. leucophaeus	FC	40.21±2.74^{d}	-	-	0.95±0.10^{gh}	-	-	20.34±5.57^{b}	6.44±0.00^{hi}	-	159.65±5.45^{e}	16.63±5.08^{ab}	27.78±0.86^{b}	**244.22**
	LS	18.14±6.04^{e}	-	-	2.35±0.00^{gh}	-	-	-	-	0.62±0.02^{d}	69.88±0.07^{f}	12.85±2.11^{b}	3.68±0.68^{c}	**103.84**
Overall QSP		**564.18**	**6.60**	**606.14**	**799.93**	**143.10**	**444.57**	**142.78**	**1243.44**	**8.69**	**2817.09**	**89.57**	**309.26**	

5. CONCLUSIONS AND RECOMMENDATIONS

This study elucidated the biological properties (antioxidant and antibacterial) and phenolic profiles [individual and total phenol/flavonoid content (TPC/TFC)] of seven *Dianthus* spp. (DAN, DAT, DBA, DCA, DKA, DLY, and DLE).

The most potent antioxidants were demonstrated by *D. balansae* and *D. aticii*. Parallel to this result, the highest TPC and TFC values have been detected in *D. balansae* and *D. aticii*, respectively. This study is the first to explore the antioxidant and TPC and TFC values of *D. leucophaeus*, *D. ancyrensis*, *D. aticii*, *D. balansae*, *D. lydus,* and *D. kastembeluensis*.

Antibacterial properties were exhibited by *D. aticii*, *D. leucophaeus, D. lydus* and *D. kastembeluensis.* The antibacterial properties of *D. kastembeluensis, D. leucophaeus*, and *D. aticii* were firstly reported.

The highest levels of rutin and phloridzin were found in *D. carmelitarum* and *D. ancyrensis*. This investigation provided the first information on the phenolic profiles of *D. kastembeluensis*, *D. aticii*, *D. leucophaeus*, and *D. lydus*.

This study's findings show the potential of the examined *Dianthus* spp. as valuable natural sources of bioactive chemicals, with uses in the pharmaceutical and nutraceutical industries. Further research is recommended to investigate the various therapeutic potentials of these species and their modes of action.

Dianthus are popular garden plants that are also used to make cut flowers, garden flowers, and dried flower arrangements. *Dianthus* species are suitable for landscaping purposes. For example, *D. kastembeluensis*, *D. lydus*, and *D. aticii* can be used in rock gardens, whilst *D. balansae* and *D. leucophaeus* can be used in Alpine gardens.

Dianthus spp.' genetic diversity and natural habitats must be preserved through biodiversity conservation efforts in order to use them medicinally and economically in the future.

6. REFERENCES

"Vancouver citation system was used in this thesis."

1. Tatlı Çankaya İ. Bitkiden ilaca Tarihi yolculuk ve fitoterapide temel Kavramlar. Fitoterapi Uygulama Kitabı. Sağlık Bakanlığı Sağlık Hizmetleri Genel Müdürlüğü, Sağlık Bakanlığı. 1234. T.C. Sağlık Bakanlığı Sağlık Hizmetleri Genel Müdürlüğü GETAT Daire Bakanlığı; 2022; 4-20.

2. Kendir G, Güvenç A. Etnobotanik ve Etnobotanik Çalışmalara Genel Bir Bakış. Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi. 2010;30(1):49-80.

3. Baytop T. Türk Eczacılık Tarihi Kitabı. İstanbul: İstanbul Üniversitesi Yayınevi; 1985.

4. Baytop T. Türkiye'de Bitkiler ile Tedavi. Geçmişte ve Bugün. 2. Baskı. İstanbul: Nobel Tıp Kitabevleri; 1999.

5. Ertuğ F. Etnobotanik. In: Güner A, editor. Resimli Türkiye Florası - Cilt 1. İstanbul: ANG Vakfı Nezahat Gökyiğit Botanik Bahçesi Yayınları; 2014.

6. Gurnani N, Mehta D, Gupta M, Mehta BK. Natural Products: Source of Potential Drugs. Afr J Basic Appl Sci. 2014;6(6):171-186.

7. Baytop T. Therapy with medicinal plants in Turkey (past and present). Istanbul University Publications. No. 3225/40. Istanbul (in Turkish); 1984.

8. Bulut G, Tuzlacı E, Doğan A, Senkardes I. An Ethnopharmacological review on the Turkish Apiaceae species. J Fac Pharm Istanbul. 2014;44(2):163-179.

9. Aslan A, Mat A, Ozhatay N, Sariyar G. A Contribution to Traditional Medicine in West Anatolia. J Fac Pharm Istanbul. 2007;39:73-83.

10. Baytop A. 1829-1960 yılları arasında İstanbul'da basılmış Farmasötik Botanik Ders Kitapları. Mar. Üniv. Ecz. Der. 1992;8(1):65-84.

11. Böttger S, Melzig MF. Triterpenoid saponins of the Caryophyllaceae and Illecebraceae family. Phytochemistry Letters. 2011;4:59-68.

12. The Angiosperm Phylogeny Group. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society. 2009;161:105-121.

13. Davis PH. Caryophyllaceae. In: Davis PH, ed. Flora of Turkey and the East Aegean Islands. Vol. 2. Edinburgh University Press; 1967:15.

14. Jia Z, Koike K, Sahu NP, Nikaido T. Triterpenoid saponins from the Caryophyllaceae family. Stud Nat Prod Chem. 2002;26:3-61.

15. Mamadalieva NZ, Ulchenko NT, Yuldasheva NK, Zhanibekov AA, Egamberdieva DR, Glushenkova AI. Neutral lipids and biological activity of the CHCI3 extract of the aerial part of *Silene guntensis*. Chemistry of Natural Compounds. 2010;46:94-96.

16. Cullen J, Coode MJE. Flora of Turkey and the East Aegean Islands, Volume 2. In: Davis PH, editor. Vol. 2. Edinburgh University Press; 1967. Available from: http://www.jstor.org/stable/10.3366/j.ctvxcrd1p.

17. Akinrinde A, Van de Venter M, Koekemoer T, Bradley G. Anti-proliferative activities of the aqueous root extract of *Dianthus thunbergii* SS Hooper (Caryophyllaceae). Afr J Tradit Complement Altern Med. 2018;15:32-44.

18. Hamzaoglu E, Koc M, Buyuk I, Aksoy A, Soydam Aydin S. Presence of *Dianthus roseoluteus* Velen. (Caryophyllaceae) in Turkey and a new species: *Dianthus macroflorus* Hamzaoglu. Syst Bot. 2015;40:208-213. https://doi.org/10.1600/036364415x686512.

19. Shishkin BK. Dianthus L. In: Flora of the USSR: Centrospermae, Volume 6, Shishkin BK, editor. Isdatel stvo Akademii Nauk SSSR, Moscow, Russia; 1970. pp. 643-644.

20. Singh B, Chaurasia OP, Jadhav KL. An ethnobotanical study of Indus valley (Ladakh). J Econ Taxonomic Bot. Additional Series. 1996;12:92–101.

21. Sargın SA, Akçicek E, Selvi S. An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. J Ethnopharmacol. 2013;150:860–874. https://doi.org/10.1016/j.jep.2013.09.040.

22. Chandra S, Rawat DS. Medicinal plants of the family Caryophyllaceae: a review of ethno-medicinal uses and pharmacological properties. Integr Med Res. 2015;4:123–131. https://doi.org/10.1016/j.imr.2015.06.004.

23. Chandra S, Rawat D, Chandra D, Rastogi J. Nativity, phytochemistry, ethnobotany and pharmacology of Dianthus caryophyllus. Res J Med Plant. 2016;10:1–9. https://doi.org/10.3923/rjmp.2016.1.9.

24. Bafier KHC. Plants used as analgesic in the folk medicine of Turkey. In Proceedings of the IVth International Congress of Ethnobotany (ICEB 2005). 2006;167–171.

25. Bafier KHC, Tümen G, Malyer H, Kırımer N. Plants used for common cold in Turkey. In Proceedings of the IVth International Congress of Ethnobotany (ICEB 2005). 2006;133–137.

26. Çömlekçioğlu N, Karaman Ş. Kahramanmaraş şehir merkezindeki aktarlarda bulunan tıbbi bitkiler. KSÜ Fen ve Mühendislik Dergisi. 2008;11:23–32.

27. Patharaj J, Kannan R. Medicinal plants used by Thoriya ethnic (Sub-tribe of Baduga) in Nilgiris, India. J Med Plants. 2017;5:30–38. https://doi.org/10.13140/rg.2.2.18406.47682.

28. Moteetee A, Van Wyk BE. The medical ethnobotany of Lesotho: A review. Bothalia. 2011;41:209–228. https://doi.org/10.4102/abc.v41i1.52.

29. Moteetee A, Moffett R, Seleteng-Kose L. A review of the ethnobotany of the Basotho of Lesotho and the Free State Province of South Africa (South Sotho). S Afr J Bot. 2019;122:21–56. https://doi.org/10.1016/j.sajb.2017.12.012.

30. Kose LS, Moteetee A, Van Vuuren S. Ethnobotany, toxicity and antibacterial activity of medicinal plants used in the Maseru District of Lesotho for the treatment of selected infectious diseases. S Afr J Bot. 2021;143:141–154. https://doi.org/10.1016/j.sajb.2021.07.048.

31. Guarino C. Ethnobotanical study of the Sannio area, Campania, Southern Italy. Ethnobot Res Appl. 2008;6:255. https://doi.org/10.17348/era.6.0.255-317.

32. Sekharan R, Jagadeesan M. An ethnobotanical survey of Javvadhu Hills, Tamil Nadu. Anc Sci Life. 1997;16:206–214.

33. Pieroni A, Quave CL, Villanelli ML, Mangino P, Sabbatini G, Santini L, Tomasi M. Ethnopharmacognostic survey on the natural ingredients used in folk cosmetics, cosmeceuticals, and remedies for healing skin diseases in the inland Marches, Central-Eastern Italy. J Ethnopharmacol. 2004;91:331–344. https://doi.org/10.1016/j.jep.2004.01.015.

34. Deniz L, Serteser A, Kargıoğlu M. Uşak Üniversitesi ve yakın çevresindeki bazı bitkilerin mahalli adları ve etnobotanik özellikleri. Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi. 2010;10:57–72.

35. Jerves-Andrade L, León-Tamariz F, Peñaherrera E, Cuzco N, Tobar V, Ansaloni R, Wilches I. Medicinal plants used in South Ecuador for gastrointestinal problems: An evaluation of their antibacterial potential. J Med Plants Res. 2014;8:1310–1320. https://doi.org/10.5897/JMPR2014.5656.

36. Macía MJ, García E, Vidaurre PJ. An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol. 2005;97:337–350. https://doi.org/10.1016/j.jep.2004.11.022.

37. Ferreira EC, Anselmo M, Guerra N, Lucena C, Felix C, Bussmann R, Paniagua-Zambrana N, Lucena R. Local knowledge and use of medicinal plants in a rural community in the agreste of Paraíba. Evid Based Complement Alternat Med. 2021;2021:1–16. https://doi.org/10.1155/2021/9944357.

38. Khatib C, Nattouf A, Agha MIH. Ethnobotanical survey of medicinal herbs in the Western region in Syria (Latakia and Tartus). Preprint available at Research Square. 2021. https://doi.org/10.21203/rs.3.rs-355008/v1.

39. Asaadi AM. Study of medicinal herbs Used by Kurmanjis Kurdish of Bojnourd in North Khorasan province of Iran. J Ethno-Pharmaceutical Products. 2021; 2:21–34.

40. Yüzbaşıoğlu E. Reşadiye (A6, Tokat, Türkiye) ve çevresinin etnobotaniği/The ethnobotany of Reşadiye (A6, Tokat, Turkey) and it's environs. Çanakkale Onsekiz Mart Univ, Inst Sci (MSc thesis). 2010.

41. Chooi OH. Ethnophytotherapies for Treatment of and Prevention against Cancer. Plant Resources. 1999;2:49–58. https://doi.org/10.1016/j.toxlet.2004.05.011.

42. Weckerle CS, Ineichen R, Huber FK, Yang Y. Mao's heritage: medicinal plant knowledge among the Bai in Shaxi, China, at a crossroads between distinct local and common widespread practice. J Ethnopharmacol. 2009;123:213–228. https://doi.org/10.1016/j.jep.2009.03.014.

43. Bağcı Y. Sarıveliler (Karaman) ve çevresinde yetişen bitkilerin etnobotanik özellikleri. Selçuk Üniv Fen Fak Fen Derg. 2016;42:84–107.

44. Kilic M, Yıldız K, Kılıç FM. Traditional uses of wild plants in Mardin central district and attached villages (Turkey). Indian J Tradit Knowl. 2021;20:784–798. https://doi.org/10.56042/ijtk.v20i3.31160.

45. Ahmadipour S, Mohsenzadeh A, Ahmadipour S, Eftekhari Z, Tajeddini P. Ethnobotanical identification of medicinal plants effective on toothache in Shiraz, South Iran. Der Pharmacia Lettre. 2015;7:419-426.

46. Nawash O, Shudiefat M, Al-Tabini R, Al-Khalidi K. Ethnobotanical study of medicinal plants commonly used by local Bedouins in the Badia region of Jordan. J Ethnopharmacol. 2013;148:921–925. https://doi.org/10.1016/j.jep.2013.05.044.

47. Menković N, Šavikin K, Tasić S, Zdunić G, Stešević D, Milosavljević S, Vincek D. Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). J Ethnopharmacol. 2011;133:97–107. https://doi.org/10.1016/j.jep.2010.09.008.

48. Van Wyk BE, De Wet H, Van Heerden FR. An ethnobotanical survey of medicinal plants in the southeastern Karoo, South Africa. S Afr J Bot. 2008;74:696–704. https://doi.org/10.1016/j.sajb.2008.05.001.

49. Ghasemi PA, Momeni M, Bahmani M. Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abdanan districts, Ilam province, Iran. Afr J Tradit Complement Altern Med. 2013;10:368–385. https://doi.org/10.4314/ajtcam.v10i2.24.

50. Asong JA, Ndhlovu PT, Khosana NS, Aremu AO, Otang-Mbeng W. Medicinal plants used

for skin-related diseases among the Batswanas in Ngaka Modiri Molema District Municipality, South Africa. S Afr J Bot. 2019;126:11–20. https://doi.org/10.1016/j.sajb.2019.05.002.

51. Yıldırım Hİ. Alanya ve Gazipaşa (Antalya)da halk tarafından kullanılan bazı doğal bitkilerin etnobotanik özellikleri/The ethnobotanic features of some natural herbs used by the people in Alanya and Gazipaşa (Antalya). Afyon Kocatepe University, Institute of Science (MSc thesis). 2015.

52. Jafari Footami I, Akbarlou M. Traditional and local use of medicinal plants by local communities in Hezar Jerib summer area, north of Iran. J Med Herbs. 2017;8:27–39. https://doi.org/10.18869/JHD.2017.27.

53. Akan H, Korkut MM, Balos MM. Arat Dağı ve çevresinde (Birecik, Şanlıurfa) etnobotanik bir araştırma. Fırat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi. 2008;20:67-81.

54. Daoud RTE. Studies on folkloric medicinal plants used by Palestinians in the Qalqilia district (Doctoral dissertation).

55. Akyol Y, Altan Y. Ethnobotanical studies in the Maldan Village (Province Manisa, Turkey). Marmara Pharm J. 2013;17:21-25. https://doi.org/10.1016/j.jep.2013.09.040.

56. Chaudhary MI, He Q, Cheng YY, Xiao PG. Ethnobotany of medicinal plants from Tian Mu Shan biosphere reserve, Zhejiang-province, China. Asian J Plant Sci. 2006;5:646-653. https://doi.org/10.3923/ajps.2006.646.653.

57. Tariq A, Sadia S, Pan K, Ullah I, Mussarat S, Sun F, et al. A systematic review on ethnomedicines of anti-cancer plants. Phytother Res. 2017;31(2):202-264.

58. Bussmann RW, Batsatsashvili K, Kikvidze Z. Dianthus superbus L., Dianthus sp. Caryophyllaceae. Ethnobotany of the Mountain Regions of Central Asia and Altai. 2020;1–3. https://doi.org/10.1007/978-3-319-77087-1_49-1.

59. Rigat M, Bonet M À, Garcia S, Garnatje T, Vallès J. Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula). J Ethnopharmacol. 2007;113:267–277. https://doi.org/10.1016/j.jep.2007.06.004.

60. Oyedemi SO, Bradley G, Afolayan AJ. Ethnobotanical survey of medicinal plants used for the management of diabetes mellitus in the Nkonkobe municipality of South Africa. J Med Plants Res. 2009;3:1040–1044.

61. Nacakcı F, Dutkuner İ. A study of ethnobotany in Kumluca (Antalya). Turk J Forestry. 2018;19:113–119. https://doi.org/10.18182/tjf.421970.

62. Arı S, Temel M, Kargıoğlu M, Konuk M. Ethnobotanical survey of plants used in Afyonkarahisar-Turkey. J Ethnobiol Ethnomed. 2015;11:1–15. https://doi.org/10.1186/s13002-015-0067-6.

63. Eşen B. Aydınlar Köyü ve çevresinin (Erdemli/Mersin) etnobotanik özellikleri/The ethnobotanical properties of Aydinlar Village and it's vicinity (Erdemli/Mersin). Selçuk University, Institute of Science (MSc thesis). 2008.

64. Saday H. Güzeloluk Köyü ve çevresinin (Erdemli/Mersin) etnobotanik özellikleri/Ethnobotanical properties of Güzeloluk Village and its vicinity (Erdemli/Mersin). Selçuk University, Institute of Science (MSc thesis). 2009.

65. Erbay MŞ, Sezin A, Melikoğlu G. Plants used as painkillers in folk medicine in Turkey IV–Toothache. İstanbul J Pharm. 2018;48:49–54.

66. Bağcı Y, Keskin L. An ethnobotanical field survey in the Kadınhanı district of Konya in Turkey. Kahramanmaraş Sütçü İmam Üniv Tarım ve Doğa Derg. 2022;25:312–336. https://doi.org/10.18016/ksutarimdoga.vi.865880.

67. Sargin SA, Selvi S, Lopez V. Ethnomedicinal plants of Sarigöl district (Manisa), Turkey. J Ethnopharmacol. 2015;171:64–84. https://doi.org/10.1016/j.jep.2015.05.031.

68. Azizi H, Keshavarzi M. Ethnobotanical study of medicinal plants of Sardasht, Western Azerbaijan, Iran. J Med Herbs. 2015;6:113–119.

69. Tong Y, Luo JG, Wang R, Wan XB, Kong LY. New Cyclic Peptides with Osteoblastic Proliferative Activity from *Dianthus superbus*. Bioorg Med Chem Lett. 2012;22:1908-1911.

70. Yu JO, Liao ZX, Lei JC, Hu XM. Antioxidant and cytotoxic activities of various fractions of ethanol extract of *Dianthus superbus*. Food Chem. 2007;104:1215–1219. https://doi.org/10.1016/j.foodchem.2007.01.039.

71. Gou J, Zou Y, Ahn J. Enhancement of antioxidant and antimicrobial activities of *Dianthus superbus*, *Polygonum aviculare*, *Sophora flavescens*, and *Lygodium japonicum* by pressure-assisted water extraction. Food Sci Biotechnol. 2011;20:283–287. https://doi.org/10.1007/s10068-011-0040-7.

72. Hikino H, Ohsawa T, Kiso Y, Oshima Y. Analgesic and Anthihepatotoxic Actions of Dianosides, Triterpenoid Saponins of *Dianthus superbus* var. *longicalycinus* Herbs. Planta Medica. 1984;51:353–355.

73. Oshima Y, Oshawa T, Oikawa K, Konno C, Hikino H. Structures of Dianosides A and B, Analgesic Principles of *Dianthus superbus* var. *longicalycinus* Herbs. Planta Medica. 1984a;50(1):40-43.

74. Bonjar S. Antibacterial screening of plants used in Iranian folkloric medicine. Fitoterapia. 2003;75:231–235.

75. Mohammed MJ, Al-Bayati FA. Isolation and identification of antibacterial compounds from *Thymus kotschyanus* aerial parts and *Dianthus caryophyllus* flower buds. Phytomedicine. 2009;16:632–637. https://doi.org/10.1016/j.phymed.2008.12.026.

76. Galeotti F, Barile E, Curir P, Dolci M, Lanzotti V. Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. Phytochemistry Letters. 2008;1:44–48.

77. Martineti V, Tognarini I, Azzari C, Sala SC, Clematis F, Dolci M, Raimondi L, Scaglianti M, Bandiera E, Braca A, Curir P. Inhibition of in vitro growth and arrest in the G0/G1 phase of HCT8 line human colon cancer cells by kaempferide triglycoside from *Dianthus caryophyllus*. Phytother Res. 2010;24:1302–1308. https://doi.org/10.1002/ptr.3105.

78. Durucasu I, Mutlu K, Şik L, Yaşa İ, Arda N, Kirmizigul S. Apolar constituents of some biologically active *Dianthus* species from Western Anatolia. Chem Nat Compd. 2009;45:782–785. https://doi.org/10.1007/s10600-010-9506-8.

79. Erturk O. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. Biologia. 2006;61(3):275–278.

80. Gevrenova R, Joubert O, Mandova Tsv, Zaiou M, Chapleur Y, Henry M. Cytotoxic effects of four Caryophyllaceae species extracts on macrophage cell lines. J Pharm Biol. 2014;52(7):919–925.

81. Hsu HY, Chen YP, Shen CS, Hsu CC, Chang HC. Oriental Materia Medica. Long Beach, Calif, USA: Oriental Healing Arts Institute; 1986.

82. Kosuge T, Yokota M, Sugiyama K. Studies on antitumor activities and antitumor principles of Chinese herbs. I. Antitumor activities of Chinese herbs. Yakugaku Zasshi. 1985;105(8):791–795.

83. Kim SY, Kim JH, Kim SK, Oh MJ, Jung MY. Antioxidant activities of selected oriental herb extracts. Journal of the American Oil Chemists' Society. 1994;71(6):633–640.

84. Casiglia S, Bruno M, Senatore F. Volatile constituents of *Dianthus rupicola* Biv. from

Sicily: activity against microorganisms affecting cellulosic objects. Natural Product Research. 2014;28(20):1739-1746.

85. eFloras. Published on the Internet http:/ /www.efloras.org [accessed 03 May 2024] *" Missouri Botanical Garden, St. Louis, MO and Harvard University Herbaria, Cambridge, MA. 2008.

86. Google Earth Version 10.56.0.1. Google; https://earth.google.com/static/multithreaded/versions/10.56.0.1/index.html [accessed 30 June 2024].

87. Reeve H. *Dianthus* L. In: Davis PH, editor. Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press; 1967. p. 99.

88. Hamzaoğlu E, Koç M, Aksoy A. *Dianthus aticii*, a new species from Turkey (Caryophyllaceae). PhytoKeys. 2015;(48):21.

89. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-1200.

90. Turker AU, Yildirim AB, Tas I, Ozkan E, Turker H. Evaluation of some traditional medicinal plants: phytochemical profile, antibacterial and antioxidant potentials. Rom Biotechnol Lett. 2021;26:2499–2510. http://doi.org/10.25083/rbl/26.2/2499.2510.

91. Turker AU, Yildirim AB, Tas I, Ozkan E, Turker H. Evaluation of some traditional medicinal plants: phytochemical profile, antibacterial and antioxidant potentials. Rom Biotechnol Lett. 2021;26:2499–2510. http://doi.org/10.25083/rbl/26.2/2499.2510.

92. Bauer A, Kirby W, Sherris J, Turk M. Antibiotic susceptibility testing by a standard single disc diffusion method. Am J Clin Pathol. 1966;45(4):493-496.

93. Andrews J. BSAC standardized disc susceptibility testing method (version 8). J Antimicrob Chemother. 2009;64:454–489. https://doi.org/10.1093/jac/dkp244.

94. Celik AK, Usta NC, Baba Y, Cimen A, Turker AU. Phenolic characterization, antimutagenic, antioxidant and antibacterial capacities of seven endemic *Dianthus* species from Turkey. South African Journal of Botany. 2024 Jan;164:39-49.

95. Birben E, Sahiner U, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9–19. https://doi.org/10.1097/wox.0b013e3182439613.

96. Gülcin I. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012;86:345-391.

97. Zucca P, Sanjust E, Trogu E, Sollai F, Rescigno A. Evaluation of antioxidant capacity of antioxidant-declared beverages marketed in Italy. Ital J Food Sci. 2010;22:313–319.

98. Aliyazıcıoğlu R, Demir S, Badem M, Sener SO, Korkmaz N, Demir E, Özgen U, Alpay Karaoğlu Ş, Aliyazıcıoğlu Y. Antioxidant, antigenotoxic, antimicrobial activities and phytochemical analysis of *Dianthus carmelitarum*. Records Nat Prod. 2017;11:270–284.

99. Turan I, Demir S, Aliyazicioglu R, Kilinc K, Ozer Yaman S, Akbulut Cakiroglu K, Kanbolat S, Ayazoglu Demir E, Mentese A, Aliyazicioglu Y, Deger O. Dimethyl sulfoxide extract of *Dianthus carmelitarum* induces S phase arrest and apoptosis in human colon cancer cells. Nutr Cancer. 2019;71:1181–1188. https://doi.org/10.1080/01635581.2019.1598563.

100. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157–2184. https://doi.org/10.1016/j.lfs.2003.09.047.

101. Kazeem MI, Ashafa AOT. In-vitro antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. J Herb Med. 2015;5:158–164.

https://doi.org/10.1016/j.hermed.2015.06.003.

102. Benli M, Bingöl Ü, Geven F, Güney K, Yigit N. Antimicrobial activity of some endemic plant species from Turkey. Afr J Biotechnol. 2007;6:1774–1778. https://doi.org/10.5897/ajb2007.000-2260.

103. Hürkül MM, Kıymacı ME. Türkiye'deki bazı endemik bitkilerin antibakteriyel aktivitesinin incelenmesi (Study of the antibacterial activity of some endemic plants of Turkey). In VI. Uluslararasi Battalgazi Bilimsel Çalışmalar Kongresi. 2021. pp. 231–236.

104. Lamula SN, Ashafa AT. Antimicrobial and cytotoxic potential of *Dianthus basuticus* used in Basotho traditional practice. Bangladesh J Pharmacol. 2014;9:105–111. https://doi.org/10.3329/bjp.v9i1.17530.

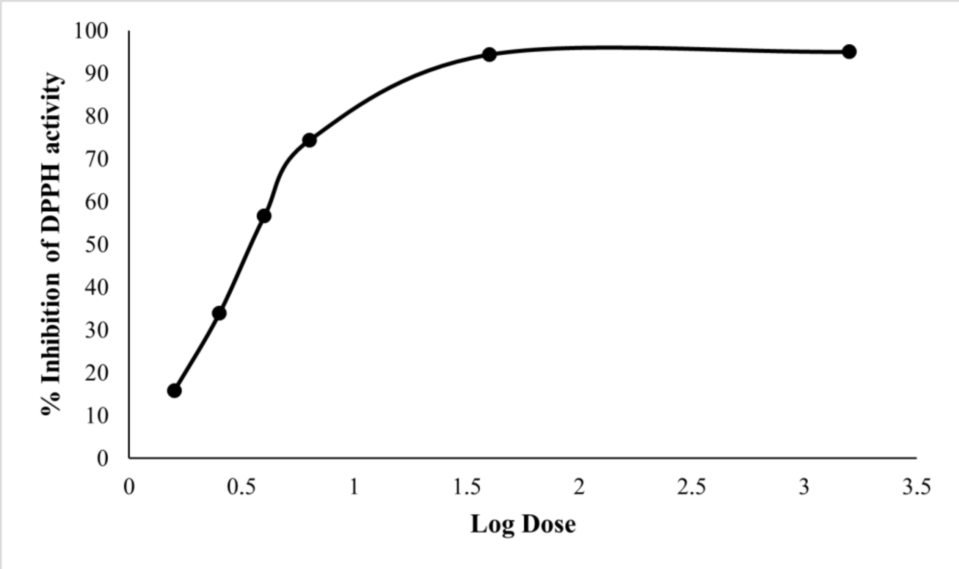
105. Dahiya R. Synthesis and biological activity of a cyclic hexapeptide from *Dianthus superbus*. Chem Papers. 2008;62:527-535. https://doi.org/10.2478/s11696-008-0052-9.

106. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J. 2017;25:149–164. https://doi.org/10.1016/j.jsps.2016.04.025.

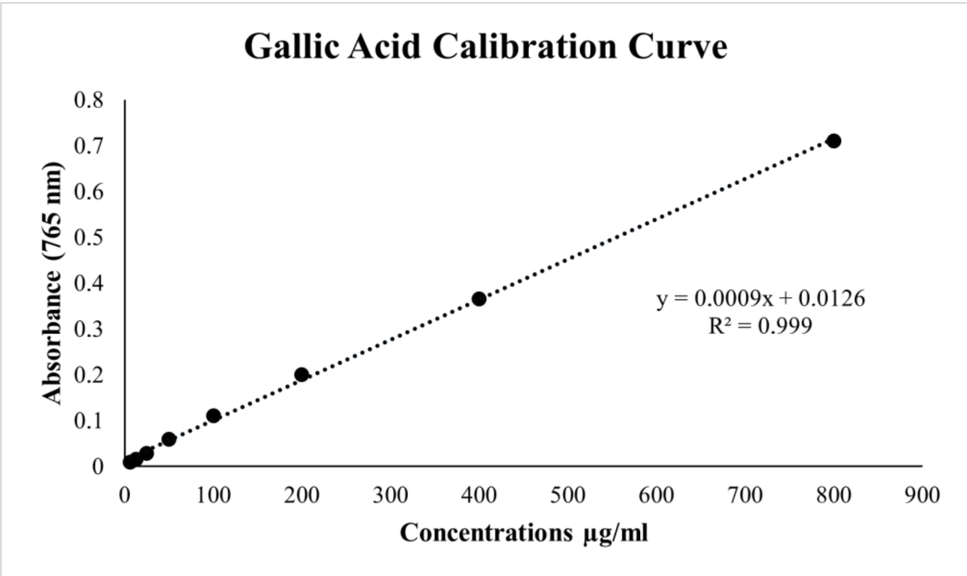
107. Tian L, Cao J, Zhao T, Liu Y, Khan A, Cheng G. The bioavailability, extraction, biosynthesis and distribution of natural dihydrochalcone: phloridzin. Int J Mol Sci. 2021;22:962. https://doi.org/10.3390/ijms22020962.

108. Meng S, Cao J, Feng Q, Peng J, Hu Y. Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. Evid Based Complement Alternat Med. 2013;2013:801457. https://doi.org/10.1155/2013/801457.

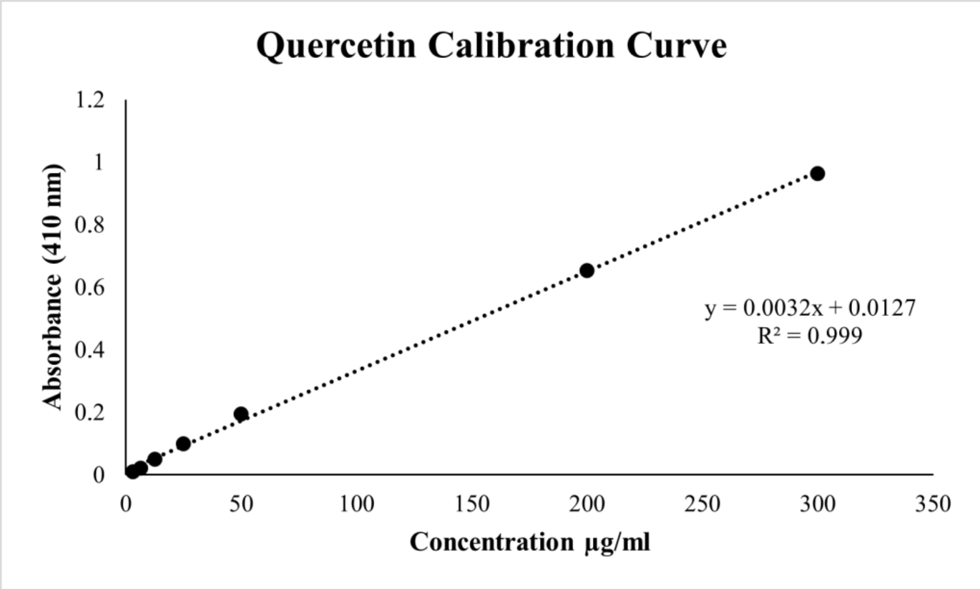
7. APPENDICES



Apendix 1. IC50 value of quercetin standard for DPPH free radical scavenging effect of *Dianthus* treatments



Apendix 2. Calibration curve for gallic acid standard of *Dianthus* treatments.



Apendix 3. Calibration curve for quercetin standard of *Dianthus* treatments.