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Evaluation of Antimicrobial Effects of Some Plant Species Growing in Turkiye: *Verbascum lydium* Boiss. var. *Lydium Boiss.*, *Euphorbia anacampseros* Boiss. var. *tmolea* M.S. Khan., *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn., *Stachys tmolea* Boiss. subsp. *tmolea* and *Aronia melanocarpa* (Michx.) Elliott

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Abstract
The increasing prevalence of antibiotic-resistant bacteria has emerged as a critical global public health issue, necessitating the exploration of alternative measures to combat infectious diseases. Traditional medicinal plants have been long recognized for their potential in providing natural compounds with antimicrobial properties for various therapeutic purposes. The diverse plant biodiversity in Turkiye offers a promising potential for the discovery of novel antimicrobial compounds. Therefore, this study aimed to investigate the antimicrobial activities of extracts from several endemic and non-endemic plant species against various microorganisms. The endemic species included *Verbascum lydium* Boiss. var. *lydium* Boiss. (stem), *Euphorbia anacampseros* Boiss. var. *tmolea* M. S. Khan. (root), *Stachys tmolea* Boiss. subsp. *tmolea* (leaves and stem), *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn. (fruit, petiole, root, leaves), while non-endemic species were represented by cultivated *Aronia melanocarpa* (Michx.) Elliott (fruit). In this research, the effectiveness of *V. lydium* var. *lydium* (stem), *E. anacampseros* var. *tmolea* (root), *R. pisiformis* subsp. *pisiformis* (all tested parts) and *A. melanocarpa* (fruit) were demonstrated against *Staphylococcus aureus* and *Escherichia coli*. Methanolic extracts of the roots and leaves of *R. pisiformis* subsp. *pisiformis* showed the most promising bioactivity with a MIC value of 62.5 µg mL^{-1} on *Pseudomonas aeruginosa* among all tested extracts. In addition, the results showed that antimicrobial activities were observed for *V. lydium*; *S. tmolea* and *R. pisiformis* against *Candida parapsilosis*, *Candida glabrata* and *Klebsiella pneumoniae* with MIC values of 31.5 µg mL^{-1} per each. The findings of this study could provide valuable information for future research into the use of Turkish plants for pharmacological purposes, public health and traditional medicine applications.
Keywords: *Verbascum lydium*, *Euphorbia anacampseros*, *Rosa pisiformis*, *Aronia melanocarpa*, *Stachys tmolea*, antimicrobial.

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Türkiyede yetişen bazı bitki türlerinin antimikrobiyal etkilerinin değerlendirilmesi: *Verbascum lydium* Boiss. var. *lydium* Boiss., *Euphorbia anacampseros* Boiss. var. *tmolea* M.S. Khan., *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn., *Stachys tmolea* Boiss. subsp. *tmolea* ve *Aronia melanocarpa* (Michx.) Elliott

Antibiyotiklere dirençli bakterilerin yaygınlığının artması sebebiyle, bulaşıcı hastalıklarla mücadele etmek için alternatif yöntemlerin araştırılması, halk sağlığı açısından kritik öneme sahip bir durum haline gelmiştir. Geleneksel tıbbi bitkiler, çeşitli terapötik amaçlar için kullanılabilecek doğal antimikrobiyal özelliklere sahip bileşikler içermesi ile bilinmektedir. Türkiye'deki bitki çeşitliliği, yeni antimikrobiyal bileşiklerin keşfi için umut verici bir potansiyel sunmaktadır. Bu araştırma, bazı bitki türlerinin ekstrelerinin antimikrobiyal aktivitelerini araştırmayı amaçlamıştır. Endemik türler arasında *Verbascum lydium* var. *lydium* Boiss. (sap), *Euphorbia anacampseros* var. *tmolea* Boiss. (kök), *Stachys tmolea* Boiss. subsp. *tmolea* (yapraklar ve sap), *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn. (meyve, sap, kök, yaprak) bulunurken, endemik olmayan tür *Aronia melanocarpa* (Michx.) Elliott (meyve) ile temsil edilmiştir. *V. lydium* var. *lydium*, *E. anacampseros* var. *tmolea* (kök), *Rosa pisiformis* subsp. *pisiformis* (test edilen tüm kısımları) ve *A. melanocarpa* (meyve) örneklerinin antibakteriyel etkinlikleri *Staphylococcus aureus* ve *Escherichia coli*'ye karşı gösterilmiştir. *Rosa pisiformis*'in kökleri ve yapraklarından elde edilen metanolik ekstreler, tüm test edilen ekstreler arasında *Pseudomonas aeruginosa* üzerinde 62.5 µg mL^{-1} MIC değeriyle en umut verici biyoaktiviteyi göstermiştir. Ayrıca, *Verbascum lydium* var. *lydium*; Stachys tmolea subsp. tmolea ve *Rosa pisiformis* subsp. *pisiformis* için *Candida parapsilosis*, *Candida glabrata* ve *Klebsiella pneumoniae* için antimikrobiyal aktivitelerin gözlendiği sonuçlar, her biri için 31.5 µg mL^{-1} MIC değeriyle gösterilmiştir. Bu çalışmanın bulguları, Türkiye bitkileri üzerinde gelecekte yapılacak olan farmakolojik araştırmalar ile, halk sağlığı ve geleneksel tıp uygulamaları için değerli bilgiler sağlama potansiyeline sahiptir.

Anahtar Kelimeler: *Verbascum lydium*, *Euphorbia anacampseros*, *Rosa pisiformis*, *Aronia melanocarpa*, *Stachys tmolea*, antimikrobiyal.

1. Introduction

Antibiotics have been widely used as the main therapeutic agents against bacterial and fungal infections since their discovery and were believed to lead to the eradication of infectious diseases [1]. However, the overuse or misuse of antibiotics have resulted in the emergence and spread of multi-drug resistant strains of various microorganisms, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans* causing an increase in the cost of medicines and patient mortality. Consequently, the search for new antimicrobial agents has become imperative, and attention has turned towards natural products as potential sources [2].

Many traditional medicinal plants have been used for centuries to treat infectious diseases, with some plants having demonstrated remarkable efficacy in treating infections [3]. Researchers have found that plants contain a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids, saponins and flavonoids, which exhibit *in vitro* antimicrobial properties [4]. Numerous medicinal plants are used as phytotherapeutics for treating infectious diseases due to their availability, fewer side effects, and reduced toxicity.

In this research a comprehensive screening of antimicrobial activity was performed for some plants growing in Turkiye including *Verbascum lydium* Boiss. var. *lydium* Boiss., *Euphorbia anacampseros* Boiss. var. *tmolea* M. S. Khan., *Stachys tmolea* Boiss. subsp. *tmolea*, *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn., and *Aronia melanocarpa* (Michx.) Elliott.

Verbascum is a genus of flowering plants, in the family Scrophulariaceae, being represented with approximately 360 species [5]. *V. lydium* var. *lydium*, is a subspecies of the *V. lydium* being endemic to Turkiye. In traditional medicine, various parts of the plants belonging to *Verbascum* genus have been used to treat various ailments [6]. For instance, the leaves and flowers have been used to relieve coughs, sore throat, and bronchitis [7]. The plant has also been used to treat ear infections and hemorrhoids. In addition, the seeds of the plant have been used to treat skin diseases [8]. The flowers of *V. lydium* var. *lydium* are edible and mainly consumed by children [9]. However studies on *V. lydium* var. *lydium* is quite limited. Although morphological and taxonomic studies have been conducted on this species, no study evaluating its antimicrobial effect has been found.

E. anacampseros var. *anacampseros* is an endemic plant of Turkiye belonging to the family Euphorbiaceae. This family is represented by approximately 240 genera and 6000 species worldwide. *Euphorbia* L. is the most well-known genus of the family and represented by approximately 2150 taxa worldwide, including 120 taxa in Turkiye, 18 of which are endemic to Turkiye. Studies on the bioactivity of *E. anacampseros* var. *anacampseros* are very limited, with more emphasis on botanical, morphological, and taxonomic studies [10,11]. Some phytochemical analysis was performed on the plant. According to the literature, the Fourier-transform infrared (FTIR) spectroscopic data obtained from *E. anacampseros* indicated the presence of hydrocarbons, particularly terpenoids, alkanes, and olefin structures, in the plant's composition. The efficacy of extracts with different polarities obtained from a plant against *Mycobacterium tuberculosis* has been investigated [12]. Research on bioactivity and medicinal properties of this endemic plant is very limited.

S. tmolea subsp. *tmolea* is a perennial herbaceous plant belonging to the Lamiaceae family, which is widely distributed in the Mediterranean region. The genus *Stachys* comprises over 300 species, and is known for its diverse medicinal and culinary uses. *S. tmolea* subsp. *tmolea* has a great importance for Turkiye being an endemic plant for this region. In traditional medicine, *Stachys* species has been used as a natural remedy for various ailments such as digestive problems, respiratory tract infections, and skin disorders [13]. The plant is also known for its antimicrobial, anti-inflammatory, and antioxidant properties.

In addition to its traditional use, *S. tmolea* subsp. *tmolea* has been the subject of scientific research due to its potential therapeutic benefits. Studies have shown that the plant contains

various bioactive compounds such as flavonoids, phenolic acids, and terpenes, which are responsible for its medicinal properties [14]. It has been reported that the ethanol extract of *S. tmolea* leaves exhibited strong effects against *S. aureus*, a Gram-positive strain [15]. In another research, plants were collected from the Kütahya-Eskişehir region and aerial parts were extracted using methanol and water. These extracts were analysed to determine phenolic constituents and antioxidant activities were evaluated [14].
R. pisiformis subsp. *psiformis* (Christ) D. Sosn. is a shrub species belonging to the family Rosaceae, which is widely distributed in the eastern Mediterranean region, including Turkiye, Lebanon, and Syria. In recent years, the genus has gained attention from researchers due to its potential pharmacological activities, particularly its antioxidant, antimicrobial, and anti-inflammatory properties [16]. Despite being a relatively unknown species, *R. pisiformis* has been traditionally used in local medicine for the treatment of various ailments, including gastrointestinal disorders, hemorrhoides and diabetes [17]. Ercisli et al., investigated the fatty acid composition of the seeds of the plant [18]. Also total phenolic content, ascorbic acid, total soluble solids, total dry weight, total fat, fatty acids, pH, acidity, moisture, fruit color and macro- and micro-element profiles were investigated in the fruits of *R. pisiformis* comparatively with some other *Rosa* species [19]. Yılmaz and Ercisli reported antimicrobial activity of fruits of *R. pisiformis* which was collected from the Eastern Anatolia region in Turkiye [20]. Further studies are required to fully understand the pharmacological potential of *R. pisiformis* subsp, *pisiformis* and its possible applications in modern medicine.

The present study aimed to investigate the antibacterial and antifungal activity of the extracts of endemic species *Verbascum lydium* Boiss. var. *Lydium* (stem), *Euphorbia anacampseros* Boiss. var. *tmolea* M.S. Khan (root), *Stachys tmolea* Boiss. subsp. *tmolea* (leaves and stem), *Rosa pisiformis* subsp. *pisiformis* (Christ) D. Sosn. (fruit, petiole, root, leaves) and a non-endemic species *Aronia melanocarpa* (Michx.) Elliott (fruit) against various microorganisms: *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus, Acetobacter baumannii, Pseudomonas aeruginosa, Candida albicans*, *Candida parapsilosis* and *Candida glabrata.*
This study highlights the importance of screening plants for their antibacterial and antimycotic activities. The exploration of natural products could offer a promising and sustainable approach towards the development of novel drugs. Further studies are required to determine the active compounds responsible for the observed antimicrobial activity, and to elucidate their mechanisms of action. Ultimately, the discovery of new antimicrobial agents from natural sources could have a significant impact on public health and the global fight against infectious diseases.

2. Material and Methods

2.1 Plant material

The plant materials were carefully examined to ensure their accurate identification, including their anatomical structures and physical features. The names of the species, local names, collection dates, locations, and herbarium codes are all listed in Table 1. The identification of plant species and taxa was conducted by Prof. Dr. Hatice Demiray from Ege University, Izmir, Turkiye.

Table 1. *Botanical and Depositional Characteristics of Tested Plant Species*

Extract Code	Botanical Name of plant	Part used	Collection date	Location	Herbarium Code
1	*Verbascum lydium* Boiss. var. *lydium* Boiss.	Stem	07/2017	Ödemiş-İzmir	25095
2	*Euphorbia anacampseros* Boiss. var. *tmolea* M.S. Khan	Root	07/2017	Ödemiş-İzmir	42191
3 4	*Stachys tmolea* Boiss. subsp. *tmolea*	Leaves Stem	07/2019	Ödemiş-İzmir	19578
5 6 7 8	*Rosa pisiformis* subsp. *psiformis* (Christ) D. Sosn.	Fruit Stem Root Leaves	08/2020	Akçakale-Gümüşhane	44010
9	*Aronia melanocarpa* (Michx.) Elliott	Fruit	09/2022	Büyükçekmece-İstanbul	220901

Fruits of *A. melanocarpa* (Michx.) Elliott (commonly known as chokeberries) were collected from a producer's field, in the Kamiloba region in Büyükçekmece, Istanbul during the flowering season. These fruits were cultivated and deposited at the Istanbul University-Cerrahpaşa, Faculty of Pharmacy Herbarium, with a voucher number of 220901.
The other plants tested in this study were *R. pisiformis* subsp. *psiformis* samples collected from Akçakale district of Gümüşhane province (Eastern Black Sea Region, Turkiye); *V. lydium* var. *lydium*, *E. anacampseros* var. *tmolea* and *S. tmolea* subsp. *tmolea* collected from Ödemiş-Bozdağ/İzmir and voucher specimens deposited at Ege University Herbarium Center in Izmir, Turkiye, as listed in Table 1. After collection, these plants were air-dried, ground into a fine powder, and stored in light-proof glass bottles at room temperature until extraction step.

1.2 Preparation of Herbal Extracts

Extract code 1-8: Powdered samples were macerated with methanol (10g/100mL; Fisher Scientific, analytical grade) using an ultrasonic bath for 30 minutes. Extractions were performed triplicate and then collected. The crude extracts were obtained by evaporating the organic solvents in vacuum (Heidolph Laborata, Germany).

Extract code 9: The fruits of *A. melanocarpa* were homogenized and centrifuged 3000 rpm for 3 min. Supernatant was freeze dried and stored at -20 °C until experiment.

2.3 Antimicrobial Bioactivity Test

The microbroth dilution method was utilized to determine the minimum inhibitory concentration (MIC) values of the extracts in antimicrobial activity studies following the Clinical and Laboratory Standards Institute M27-A and M27-A3 documents against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213), *A. baumannii* (ATCC 02026), *C. albicans* (ATCC 14053), *C. parapsilosis* (ATCC 22019), and *C. glabrata* (ATCC 15126) [21,22]. Stock solutions of the plant extracts were prepared at 1000 µg mL^{-1}. Density of the cells was adjusted to Mc Farland 0.5 in sterilized saline solution. Two-fold dilutions were prepared in 100 µL of Mueller-Hinton broth (Sigma-Aldrich, St. Louis, MO) and RPMI 1640

medium (Sigma-Aldrich, St. Louis, MO) for 18 hours for bacteria and yeasts. Then, 10 µL microorganisms suspensions were added for each species. The MIC values were determined visually and by spectrophotometric evaluation at 450 nm after 18 hours incubation at 37 °C. The ciprofloxacin and the fluconazole were used as reference drugs.

3. Results and Disscussion

Antimicrobial activities of nine herbal extracts (single or combine) were evaluated against *E. coli*, *P. aeruginosa*, *S. aureus*, *A. baumannii*, *C. albicans*, *C. parapsilosis* and *C. glabrata* (Table 1). In this study, the effectiveness of *V. lydium* var. *lydium*, *E. anacampseros* var. *tmolea*, *R. pisiformis* subsp. *pisiformis* and *A. melanocarpa* (fruits) were demonstrated against *S. aureus* and *E. coli* at a concentration of 62.5 µg mL^{-1} when used individually. However, *S. tmolea* subsp. *tmolea* did not exhibit any antimicrobial activity on these two species up to 125 µg mL^{-1}. Methanolic extracts of the roots and leaves of *R. pisiformis* (roots and leaves) showed the most promising bioactivity with a MIC value of 62.5 µg mL^{-1} on *P. aeruginosa* among all tested extracts (Table 2).

Table 2. *Antimicrobial activities of herbal extracts collected from Turkiye. MICs are given in µg mL^{-1}.*

Extract No	Sa	Ec	Pa	Ab	Kp	Ca	Cp	Cg
1	62.5	62.5	125	62.5	31.25	31.25	31.25	31.25
2	62.5	62.5	125	62.5	62.5	62.5	31.25	31.25
3	125	125	125	62.5	31.25	62.5	31.25	31.25
4	125	125	125	62.5	62.5	62.5	31.25	31.25
5	62.5	62.5	125	62.5	31.25	62.5	31.25	31.25
6	62.5	62.5	125	62.5	31.25	62.5	31.25	31.25
7	62.5	62.5	62.5	62.5	31.25	62.5	31.25	31.25
8	62.5	62.5	62.5	62.5	31.25	62.5	31.25	31.25
9	62.5	62.5	125	125	62.5	62.5	500	Nt
C*(5+9)	250	125	Nt	Nt	Nt	125	Nt	Nt
C*(1+2)	250	125	Nt	Nt	Nt	62.5	Nt	Nt
Ciprofloxacin	3.90	3.90	7.81	3.90	3.90	–	–	–
Fluconazole	–	–	–	–	–	1.95	3.90	7.81

1, *Verbascum lydium* var. *lydium* (stem); 2, *Euphorbia anacampseros* var. *tmolea* (stem); 3,4, *Stachys tmolea* subsp. *tmolea* (leaves and stem); 5-8, *Rosa pisiformis* subsp. *pisiformis* (fruit, petiole, root, leaves); 9, *Aronia melanocarpa* (fruit). Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Sa, *Staphylococcus aureus*; Ab, *Acetobacter baumannii*; Ca, *Candida albicans*; Cp, *C. parapsilosis*; Cg, *C. glabrata*. Nt: not tested; C*: combination formulations. Tested concentration range: 500 – 0.48 µg/mL.

All single extract treatments exhibited similar antibacterial effects at 62.5 µg mL^{-1} concentration against *A. baumannii* except for *A. melanocarpa* fruit juice for which MIC was calculated as 125 µg mL^{-1}. Striking bioactivities were observed for *V. lydium* var. *lydium; S. tmolea* subsp. *tmolea. and R. pisiformis* subsp. *psiformis* (with MIC value of 31.5 µg mL^{-1} per each) against *C. parapsilosis*, *C. glabrata* and *K. penemanue*.

The combination prepared using *E. anacampseros* var. *tmolea* roots with *V. lydium* var. *lydium* Stems displayed superior antimycotic activity on *C. albicans* compared to DMSO, the solvent control. Also, all extracts except for fruit juice of *A. melanocarpa* inhibited *Acetobacter* sp.

compared to DMSO. Methanol extract of *V. lydium* var. *lydium* stems was the most prominent preparation against *C. albicans* while only *V. lydium* var. *lydium* stem extract and *Rosa pisiformis* leaf extracts showed antimycotic activity against *C. parapsilosis.*

To our knowledge this is the first report on the antimicrobial acitivity evaluations of endemic species tested in this study. However, antibacterial effects of *A. melanocarpa* extracts against *S. aureus*, *E. coli* and *B. subtilis* were reported in the literautre [23,24]. Tanagardi investigated differential effects of commercial, liquid and dry extracts of *A. melanocarpa* on various microbial strains such as *Saccharomyces cerevisiae*, *S. aureus*, *E. coli* O157:H7, and *Salmonella typhimurium* [24]. Notably, the results demonstrated that the liquid and dry extracts of *A. melanocarpa* exhibited distinct effects on Gram (+) bacteria, Gram (-) bacteria, and the *S. cerevisiae* yeast strain. The antifungal activity of the liquid and dry extracts at a concentration of 180 µg/mL against *S. cerevisiae* resulted in a remarkable 99% reduction in fungal growth. Furthermore, the liquid extract showed significant antibacterial activity at a concentration of 180 µg/mL against *E. coli*, leading to a 99% reduction in bacterial population. In the case of *S. typhimurium*, the liquid extract at a concentration of 180 µg/mL exhibited a 100% inhibition of bacterial growth, while the dry extract did not display significant antibacterial effects. The evaluation of *S. aureus* bacterial strains further highlighted the differential antibacterial activities of the liquid and dry extracts at varying concentrations, with the liquid extract at 180 µg/mL and 90 µg/mL concentrations resulting in 100% and 99.9% bacterial reduction, respectively. The dry extract also demonstrated strong antibacterial effects, with a 99.9% inhibition of bacterial growth at a concentration of 180 µg/mL. These findings align with the result obtained in our study, where the MIC of *A. melanocarpa* was 62.5 µg/mL against both *S. aureus* and *E. coli*.

4. Conclusion

In conclusion, this study has shed light on the bioactivities of some valuable plant species in Turkiye. This provides significant contributions towards the evaluation of plants growing in Turkiye for pharmaceutical approaches, public health, traditional medicine applications, and pharmacological purposes.

Based on the findings, significant bioactivities were observed for *V. lydium* var. *lydium*, *S. tmolea* subsp. *tmolea*, and *R. pisiformis* subsp. *psiformis* (each with a MIC value of 31.5 µg mL-1) against *C. parapsilosis, C. glabrata*, and *K. penemanue*. This highlights the research value of these plants which are growing in Turkiye, emphasising their potential in exhibiting antimicrobial activity. *A. melanocarpa* also displayed antibacterial and antifungal activities consistent with finding in the literature. The obtained findings involve preliminary evaluations of tested plant species in relation to their antimicrobial potential against diverse bacterial and fungal strains. However, further research is needed to understand the mechanism of antimicrobial action of these plant extracts. The information obtained from this study serves as a starting point for future investigations, which can aid in the discovery of new drug leads and the development of novel therapeutics.

Ethics in Publishing

There are no ethical issues regarding the publication of this study. Approval from any committee is not required for collection of plant material.

Author Contributions

Authors declares the contribution of the authors is equal. Conceptualization: ÇB; Investigation: HD; Methodology: EK; Writing: ÇB and EK; Review-editing: HD.

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