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Original Article

Antimicrobial activities of some narrow endemic gypsopyhte

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ABSTRACT

Background and Aims: In this study, antimicrobial activities of extracts obtained from narrowly dispersed local endemic gypsophytes grown in extreme habitats were investigated for the first time. The aim of this study was to analyzed antimicrobial effects of narrow endemic plants that are *Thymus ekimii* Yildirimli, *Verbascum gypsicola* Vural & Aydogdu, *Glaucium secmenii* Yildirimli and *Psephellus erzincani* Wagenitz & Kandemir.
Methods: Antimicrobial activity of *T. ekimii, V. gypsicola, G. secmenii* and *P. erzincani* were determined according to the disk diffusion method. The microorganisms used for the present investigation; gram positive bacteria, gram negative bacteria and yeasts (*Bacillus megaterium* DSM32, *Escherichia coli* ATCC25922, *Candida albicans* FMC17).
Results: According to the results obtained, *P. erzincani* showed the best antimicrobial activity against *B. megaterium* DSM32 (23 mm), *E. coli* ATCC25922 (15 mm) and *C. albicans* FMC17 (23mm), respectively.
Conclusion: This study showed that extracts of these endemic plants have the potential for use as antimicrobial agents, especially *P. erzincani*.

Keywords: *Thymus ekimii, Verbascum gypsicola, Glaucium secmenii, Psephellus erzincani,* antimicrobial activity, gypsopyhte

INTRODUCTION

The increasing resistance of bacteria to clinical antibiotics necessitates the development of new agents in the treatment of diseases. Therefore, antibacterial and antifungal effects of herbal preparations are very important due to the high incidence of antibiotic resistance in treatment (Mummed, Abraha, Feyera, Nigusse, & Assefa, 2018). Some studies showed that plants can be successful in overcoming antibiotic resistance with combinatorial approaches (Van Vuuren & Viljoen, 2011; Hutchings & Cock, 2018; Blonk & Cock, 2019). Today, 80% of the active substances used in the treatment of infectious diseases are thought to be vegetable-oriented (Özdek, Seçkin, & Çibuk, 2020). Therefore, the use of plant extracts as an antioxidant and antimicrobial agent has been expanded in recent years.

Among the leading countries of the temperate zone, Turkey has a lot of floristic diversity and endemism. Therapeutic uses of plants are based on ancient times. It has been found mainly by trial and error in Anatolia as well as all over the world and medi-

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cally important plants have been widely used in traditional folk medicine for many centuries. It is known that some widespread endemic species have different antimicrobial effects (Yiğit, Kandemir, & Yiğit, 2002; Buruk, Sokmen, Aydin, & Erturk, 2006; Dulger, 2006; Benli, Güney, Bingöl, Geven, & Yiğit, 2007; Benli, Yiğit, Geven, Güney, & Bingöl, 2009; Türker, Birinci Yıldırım, Pehlivan Karakaş, & Köylüoğlu, 2009). While recent studies on antimicrobial activity are mostly concentrated on widespread endemic species, in this study, the antimicrobial effects of the gypsophyte species developing in gypseous soils, which are extremely arid habitats, were examined (Wagenitz, & Kandemir, 2008; Yıldırımlı, 2012).

T. ekimii is a member of the Lamicaceae family and it is known that this family species is used in food and cosmetics as well as in the pharmaceutical industry (Bekut et al., 2018). It is known that *Thymus* L. (thyme) is used as a spice in meals and facilitates digestion. It is used by local people for cramps, disinfection, and as an expectorant. In studies, it has been determined that there are biological activities such as antioxidant, antiseptic, and antimicrobial (Benli & Yiğit, 2005).

Some species of the genus *Verbascum* L., including *V gypsicola*, have been used extensively for centuries to treat internal and external infections. It is traditionally used by local people as a tea, and is believed to have a chest loosening and expectorant effect. In addition, some species of the *Verbascum* L. genus have biological effects such as antimalarial, antiviral, antitumor, antihepatotoxic, antihyperlipidemic, antioxidant, anti-inflammatory, antinociceptive, wound-healing, antimicrobial, anthelmintic, sedative, pre-anesthetic, and anxiolytic (Civelek, 2018).

The genus *Glaucium* Mill., which includes the species *G. secmenii*, has been used by local people for food and medical purposes. Some species' seeds, green parts, and petals are used. It is stated in some studies that it is effective in colds, bronchitis, and in conditions such as expectorant and for insomnia (Saraç et al., 2018).

There are not many studies on the antimicrobial activities of taxa belonging to the genus *Psephellus* Cass., which also includes the *P. erzincani* species, and some species have been examined for their cytotoxic, antioxidant and anti-inflammatory activities (Korkmaz et al., 2019; Demiroz, Nalbantsoy, Aydin, & Baykan, 2020).

The genus *Psephellus* has been separated from *Centaurea*, and some *Centaurea* species are used for fever, diabetes, hemorrhoids, and peptic ulcers for therapeutic purposes among people. In pharmacological and phytochemical studies, antioxidant, antimicrobial, and antipyretic properties have been determined in many different *Centaurea* species (Korkmaz et al., 2019).

In this study, it was aimed to determine the antimicrobial activities of the extracts obtained from a methanol solvent of local gypsophyte endemics *T. ekimii*, *V. gypsicola*, *G. secmenii* and *P. erzincani*.

MATERIALS AND METHODS

Collection and identification of plant material

The common feature of plants is that they are local endemic species spread on gypsum soils, which are extremely excavated arid habits for plant life. Plant materials were diagnosed using Flora of Turkey and East Aegean Islands (Ekim 2000). Identified plant samples were checked in the ANK Herbarium, and the doublet of the plants was preserved in the ANK Herbarium (Table 1).

Extract of plant material

T. ekimii, G. secmenii, V. gypsicola, P. erzincani were dried and after milling added to the 40 mL 98% methanol by weighing 1 g for each sample.

Each sample was kept on a rotary shaker at 100 rpm for 72 hours to obtain the extract. It was then filtered using Whatman filter paper and stored at 4°C for further study. Then 20 µL (500 µg/L) extracts were injected into 6 mm diameter empty antibiotic discs (Erecevit Sönmez, Kırbağ, & İnci, 2019).

Test microorganisms

In this study; *Escherichia coli* ATCC 25322 and *Bacillus megaterium* DSM32 as bacteria, and *Candida albicans* FMC17 as fungi were used. Microorganism cultures were obtained from the Fırat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection.

Preparation of microorganism cultures and testing of antimicrobial effect

The antimicrobial activity of extracts of plant samples obtained using methanol was determined according to the disk diffusion method (Collins & Lyne, 1989).

Table 1. The location and GPS coordinates and elevation of species extracted

Species	Locality, Collector and Number of plants	Elevation (m)
Thymus ekimii Yildirimli	Between Aşağıkepen and Kepen villages / Eskişehir 39°22'10.0" N 031°29 09.1" E, 05.06.2020 Kurt, L., 15214	938
Glaucium secmenii Yildirimli	Ankara-Eskişehir road side / Eskişehir 39°33'51.4" N 031°48'27.9" E, 05.06.2020 Kurt, L., 14938	988
Verbascum gypsicola Vural & Aydogdu	Beypazarı-Nallıhan road, near Çayırhan / Ankara 40°06'24.3" N 031°43'45.5" E, 06.06.2020 Kurt, L., 15583	611
Psephellus erzincani Wagenitz & Kandemir	Ilic-Divrigi road, near Bagistas village / Erzincan 39°27'02.1" N 038°28'52.4" E, 12.06.2020 Kurt, L., 14701	889

Bacterial strains (*E. coli* ATCC25322, *B. megaterium* DSM32), were incubated in Nutrient Buyyon (Difco) for 24 hours at 35±1°C and the yeast strain (*C. albicans* FMC17) was incubated at 25±1°C for 48 hours in Malt Extract Buyyon (Difco). Cultures grown in broth medium were adjusted to the 0.5 McFarland standard. The culture of prepared bacteria and yeast in broth are as follows; Mueller Hinton Agar and Yeast Malt Extract Agar were inoculated with 1% (10^6 cells/mL of bacteria, 10^4 cells/mL yeast and cells/mL as per Mc Farland standard) and after shaking well, 25 ml were placed in sterile petri dishes with a diameter of 9 cm and homogeneous dispersion was provided.

Six mm diameter antimicrobial discs (Oxoid), each of which was absorbed 20 µl of different extracts, were placed in the solidified agar medium aseptically.

After the petri dishes prepared in this way were kept at 4°C for 1.5-2 hours, the bacteria grafted plates were incubated at 37±0.1°C for 24 hours, and the yeast-grafted plates at 25±0.1°C for 72 hours.

As a control, different standard discs were used for bacteria (Piperacillin/Tozabactam 110 µg/disk) and yeasts (Mycostatin 30 µg/disk).

Inhibition zones formed on the medium at the end of the period were evaluated in mm.

RESULTS AND DISCUSSION

The antimicrobial effects of *T. ekimii*, *G. secmenii*, *V. gypsicola*, *P. erzincani* methanol extracts against *B. megaterium* DSM32, *E. coli* ATCC25322 and *C. albicans* FMC17 are shown in Table 2. Mycostatin (30 µg/disk) used for yeasts created a 15 mm inhibition zone against *C. albicans* FMC17. Piperacillin/Tozabactam (110 µg/disk) prevented the growth of tested bacteria at different rates (25-38 mm inhibition zone).

Table 2. Antimicrobial effects of *T. ekimii*, *G. secmenii*, *V. gypsicola* and *P. erzincani*.

	Diameter of Inbition Zone (mm)		
	***Bacillus megaterium* DSM32**	***Escherichia coli* 25322ATCC**	***Candida albicans* FMC17**
Thymus ekimii	11	10	14
Glaucium secmenii	11	10	12
Verbascum gypsicola	8	7	7
Psephellus erzincani	**23**	**15**	**23**
Control	25	38	15

Against *B. megaterium* DSM32 *T. ekimii, G. secmenii* and *V. gypsicola* formed a 11 mm and 8 mm zone diameter respectively, while the inhibition zone of *P. erzincani* was measured at 23 mm (Figure 1). It has been determined that *T. ekimii*, *G. secmenii*, *V. gypsicola*, *P. erzincani* prevent the development of *E. coli* ATCC25322 at different rates (7-15 mm) (Figure 2). *T. ekimii*, *G. secmenii*, *V. gypsicola* and *P. erzincani* species formed 14 mm, 12 mm, 7 mm and 23 mm inhibition zones against *C. albicans* FMC17, respectively (Figure 3). According to the results obtained, the *P. erzincani* extract showed the best antimicrobial activity against *B. megaterium* DSM32 (23 mm), *E. coli* ATCC25322 (15 mm) and *C. albicans* FMC17 (23 mm).

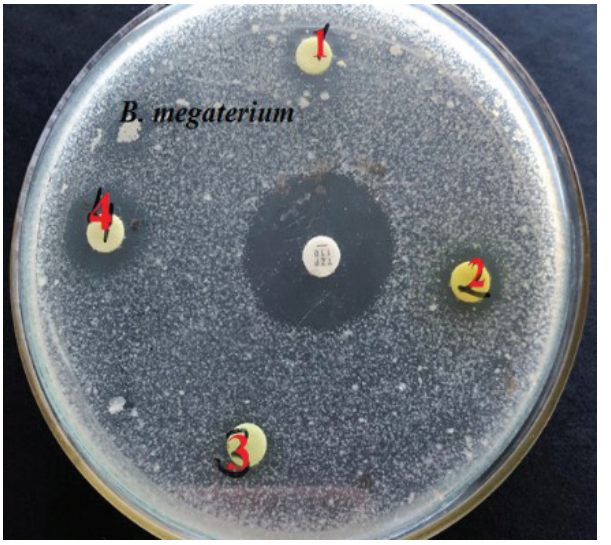

Figure 1. Antimicrobial effects of narrow endemic gypsopyhte against *B. megaterium* (numbers in petri; 1- *T. ekimii*; 2- *G. secmenii*; 3- *V. gypsicola*; 4- *P. erzincani*).

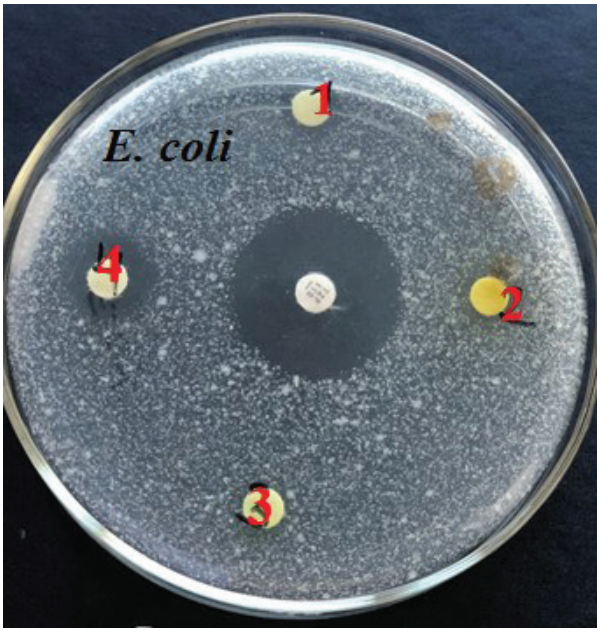

Figure 2. Antimicrobial effects of narrow endemic gypsopyhte *E. coli* (numbers in petri; 1- *T. ekimii*; 2- *G. secmenii*; 3- *V. gypsicola*; 4- *P. erzincani*).

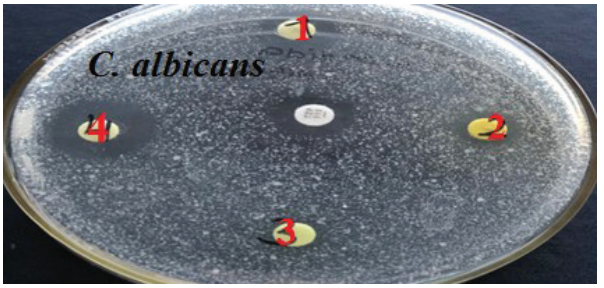

Figure 3. Antimicrobial effects of narrow endemic gypsopyhte against *C. albicans* (numbers in petri; 1- *T. ekimii*; 2- *G. secmenii*; 3- *V. gypsicola*; 4- *P. erzincani*).

The antimicrobial activity of some Thymus species have been shown in other previous studies. In the studies conducted, it was determined that essential oils obtained from *Thymus algeriensis* prevent the development of *S. aureus* ATCC25923, *B. subtilis* 166, *S. enteridis* ATCC502, *E. coli* GM109, *P. aeruginosa* and *L. monocytogynes* at different rates (9-74 mm inhibition zone) (Guesmi, Mouna, Mondher, & Ahmed, 2014). In a different study, the antimicrobial effect of essential oils of *T. vulgaris* L. against different strains of *E. coli* 25922 was determined as 22.7-2.8 µl ml^{-1}, and antimicrobial effect against different strains of *S. aureus* ATCC25923, ATCC6538 as 11.4-45.4 µl ml^{-1}. It has been reported that essential oils of the same species show 0.11 µl ml^{-1} antimicrobial activity against different strains of *C. albicans* ATTC10231 (Bogavac et al., 2015). The essential oils of *Thymus longicaulis* subsp. *longicaulis* were found to have an antimicrobial effect of 0.781 µg/ml against *S. aureus* ATCC25923 and 0.098 µg/ml against *E. coli* and *C. albicans* DSMZ1386 (Demiryapan, 2020). It was determined that *Thymus serphllum* ethanol, methanol, and water extracts did not prevent the development of *E. coli* ATCC11229 at a concentration of 150 mg/ml, and the ethanol and methanol extract prevent the development of *C. albicans* RSKK02029 (8 mm inhibition zone) (Ökmen, Arslan, Vurkun, Mammadkhanli, & Ceylan, 2017). The essential oils of *Thymus vulgaris* have been reported to have 49.27±7.26 mm against *S. aureus* NCTC8530 and 39.55±0.52 mm against *E. coli* BL21 (Kılıç, 2019).

The antimicrobial effects of methanol, ethanol, and water extracts of *Verbascum degenii* against some hospital pathogens were investigated. In the results obtained, while methanol extract formed an inhibition zone of 20±1.6 mm against *S. aureus*, ethanol extract formed an inhibition zone of 21±1.5 mm, and no inhibition zone was observed in water extract (Avşar, Keskin, & Berber, 2016).

While the water extract of *Glaucium grandiflorum* Boiss. & Huet var. *grandiflorum* has antimicrobial effects >5 mg/ml against *E. coli* ATCC25922, 0.625 mg/ml against *S. aureus* ATCC29213 and >5 mg/ml against *C. albicans* ATCC10231; ethanol extract against the same microorganisms showed antimicrobial effects >5 mg/ml, 5 mg/ml and 1.25 mg/ml, respectively (Saraç et al., 2018).

Many microorganisms that harm human health show resistance to drugs due to using unnecessary and wrong antibiotics. Therefore, there is a need to discover new substances from natural sources, including plants. In this study, the antimicrobial activity of gypsophytes grown in gypsiferous soils, which are extreme habitats for plants, were investigated. The species discussed in this study are local endemic gypsophytes and their antimicrobial activities were examined for the first time. It has been predicted that species growing in extreme habitats may have high antimicrobial activities.

Among the four gypsophytes examined in this study, *P. erzincani* extract showed the best antimicrobial activity against *B. megaterium* DSM32 (23 mm), *E. coli* ATCC25322 (15 mm) and *C. albicans* FMC17 (23 mm). Investigating the antimicrobial activities of species grown in extreme habitats may lead to new antibiotic research.

The results obtained show that antimicrobial effects of *T. ekimii*, *V. gypsicola*, *G. secmenii* and *P. erzincani* are different from *Thymus algeriensis, T. vulgaris*, *T. longicaulis* subsp. *longicaulis*, *T. serpyllum, Glaucium grandiflorum* var. g*randiflorum* in literature. As a result of the adaptation of these plants (*T. ekimii*, *V. gypsicola*, *G. secmenii* and *P. erzincani*) to extreme habitats (gypsum soils etc.), their defense systems are well developed and have a high resistance against drought, bacteria, viruses, and other pathogens. Therefore, it is very important that research of the antimicrobial effects of plant pecies living in these extreme conditions and their use as an antimicrobial agent against microorganisms receive further investigation and study.

In conclusion, these four narrow endemic plants, especially *P. erzincani,* have the potential for use as antimicrobial agents. Endemic plant species as antimicrobial agents are substantial in pharmacology and more investigation is necessary in terms of contributing to the literature.

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