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Original Article

Investigation of the antimicrobial activities of solvent extracts of two endemic species from Turkey: *Campanula tomentosa* Lam. and *Verbascum mykales* Bornm.

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ABSTRACT

Background and Aims: *Campanula tomentosa* Lam. and *Verbascum mykales* Bornm. are endemic species in Turkey. Extracts of these plants contain important natural compounds such as flavonoids, saponins and tannins. This study investigates the antimicrobial effects of leaf extracts of *C. tomentosa* and *V. mykales* against some bacteria and yeasts.

Materials and Methods: Leaves of plant samples were air-dried and ground into powder. Five solvents (ethyl acetate, methanol, acetone, chloroform, boiled water) were used for extraction. Experiments were conducted using these crude extracts on seventeen bacteria, three yeasts and three microfungi. The agar well diffusion method was used for the antimicrobial activities of the extracts. In addition, minimum inhibitory concentrations, minimum bacteriocidal concentration, minimal fungicidal concentrations were carried out.

Results: The ethyl acetate and methanol extracts of *C. tomentosa* and *V. mykales* were found to be highly effective against the tested microorganisms. According to the MIC values, the ethyl acetate extracts of *C. tomentosa* and *V. mykales* had a strong effect (4-8 µg/mL) against *Escherichia coli* ATCC 35218, *Micrococcus luteus* ATCC 9341, *Streptococcus pneumonia* ATCC 27336, *Pseudomonas aeruginosa* ATCC 35032, *Mycobacterium smegmatis* ATCC 607, *Proteus vulgaris* ATCC 33420, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778, and *Bacillus subtilis* ATCC 6633. The ethyl acetate extract of *C. tomentosa* had a moderate effect (64 µg/mL) against *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763, and the ethyl acetate extract of *V. mykales* had a moderate effect (64 µg/mL) against *Aspergillus flavus* ATCC 9807 and *Aspergillus niger* ATCC 16404. However, the boiled water extract of *C. tomentosa* and *V. mykales* had no effect on the tested microorganisms.

Conclusion: *C. tomentosa* and *V. mykales* used in the study are endemic plants and their antimicrobial activities are being investigated for the first time. The ethyl acetate extract of both plants was found to be most effective against the Gram (+) and Gram (-) bacteria. However, all extracts of both plants were found to have fewer antimicrobial effects against used yeasts and microfungi. This study demonstrates that plant extracts are more effective against prokaryotic microorganisms than eukaryotes.

Keywords: *Campanula tomentosa* and *Verbascum mykales*, antimicrobial activity, agar well diffusion, MIC/MBC/MFC

INTRODUCTION

Humans have used plants as food, spices, textiles, perfumes, and medicines for centuries. The World Health Organization (WHO) has reported that approximately 20000 plants are used as medicine. The number of plants used as medicine is estimated to be

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around 500 in Turkey (Baytop, 1999; Faydaoğlu & Sürücüoğlu, 2011; Temel, Tınmaz, Öztürk & Gündüz, 2018; Yaldız & Çamlıca, 2018). Wild plants have been used for treatment since ancient times by people living in Anatolia as in other societies (Faydaoğlu & Sürücüoğlu, 2013). The method of treatment with herbs was applied for the first time in the civilizations of Sumer, Akkad and Assyria, which were established in Mesopotamia (Dar, Shahnawaz, & Qazi, 2016). Wild plants are used as alternative treatments all over the world today. Therefore, as an alternative to synthetic drugs, interest in the use of herbal medicines has increased in the developing world. Herbs are widely used as an alternative treatment in Europe, North America and some developed countries (Keskin, 2018).

The genus Campanula belongs to the Campanulaceae family and involves 300 species (Figure 1). Many Campanula species grow in Asia, the Black Sea and the Mediterranean region. There are many endemic Campanula species in Turkey. (Ozhatay, Kultur, & Aslan, 2009). Studies on the ethnobotanical use and biological activity of various *Campanula* species have been conducted by many researchers in Turkey (Buruk, Sokmen, Aydin, & Erturk, 2006; Benli, Bingöl, Geven, Güney, & Yiğit; 2008; Tosun, Kahrıman, Çoşkunçelebi, Genç, Alpay Karaoglu, & Yaylı, 2011; Sinek, Yılmaz İskender, Yaylı, Alpay Karaoglu, & Yaylı, 2012; Usta, Birinci Yildirim, & Ucar Turker, 2014). A large number of substances from the root, stem and leaf structures of plants that can inhibit the growth of microorganisms were analyzed. The flavonoids and anthocyanins such as cyanidinand delphinidin were isolated from the *Campanula* genus. *Campanula* species have been used in folk medicine for therapy of tonsillitis, laryngitis, and bronchitis. Furthermore, they have antioxidant, antiviral, and antiallergic properties (Alhage, Elbitar, Taha, & Benvegnu, 2018; Herkul & Köroğlu, 2019).

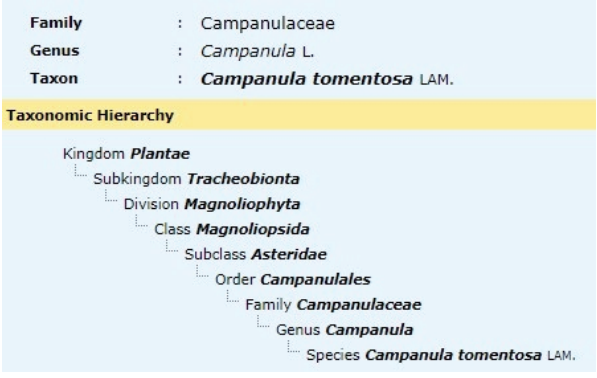

Figure 1. General taxon information of *Campanula tomentosa* Lam. (Turkish Plants Data Service) (www.tubives.com).

Verbascum belongs tothe Scrophulariaceae family and comprisesof 323 species in the world (Figure 2). The genus includes 245 species in Turkey and the endemism ratio of this genus is very high (79%) (Dulger & Dulger, 2018). The *Verbascum* species has been used as folk medicine since ancient times all over the world. Particularly, flowers and leaves of plants have been used to treat respiration disorders in phytotherapy. Ingredients of the plant, such as flavonoids, glycosides, phenylethanoids, iridoids, saponins, monoterpene and neolignans, have expectorant, diuretic and relaxing properties (Kahraman, Tatlı, Kart, Ekizoğlu, & Akdemir, 2018). Flowers containing plant phenyl porpanoids especially have anti-inflammatory effects (Karalija, Parić, Dahija, Bešta-Gajević, & Zeljković, 2018).

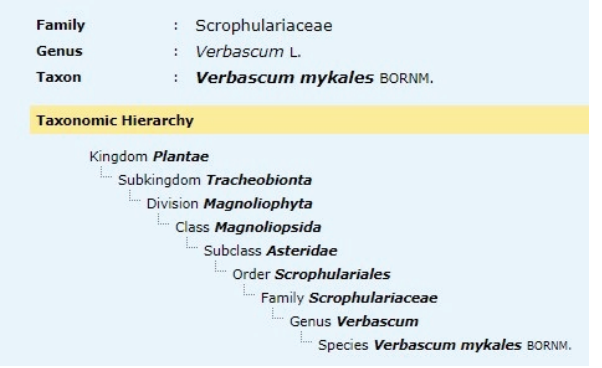

Figure 2. General taxon information of *Verbascum mykales* Bornm. (Turkish Plants Data Service) (www.tubives.com).

The antimicrobial effect of *Campanula lyrata* Lam. *subsp. lyrata* (leaf and flower) methanol extract was analyzed against *E. gallinarum* CDC-NJ-4, *E. faecalis* ATCC 29212, *B. subtilis* RSHI, *E. coli* RSHI, *Shigella* sp., *E. coli* ATCC 25922, *S. pyogenes* ATCC 19615, *S. aureus* ATCC 29213, *L. monocytogenes* ATCC 7644, *P. aeruginosa* ATCC27853, *S. cerevisiae* (Pakmaya), *C. albicans* 845981, *C. crusei* ATCC 6258 and *C. albicans* 900628. It was revealed that *C. lyrata subsp. lyrata* extract was effective against *B. subtilis* and *S. aureus*. The minimum inhibitory concentration of *C. lyrata subsp. lyrata* extract was determinated as 29 mg/mL for *B. subtilis* and 14.5 mg/mL for *S. aureus* (Benli, Bingol, Geven, Guney & Yigit, 2008). Antimicrobial activities of the dichloromethane, ethanol: water (70:30 v/v), water, and methanol extracts of *Verbascum macrurum* Ten. leaves were examined and it was demonstrated that the ethanol: water extract was the most effective (Guarino, 2002). The ethanolic extract of *V. qulebrium* Boiss. was tested against *S. aureus*, *S. typhi*, *S. pastorianus*, *E. coli*, *B. subtilis* and *P. aeruginosa* and the best inhibition effect was obtained against the Gram (+) bacteria *B. subtilis* and the yeast *S. pastorianus* (Khafagi, 2001). Antimicrobial effect of the extracts of *V. olympicum* Boiss., *V. prusianum* Boiss. and *V. bombyciferum* Boiss. were evaluated against Gram (+) and Gram (-) bacteria, and yeasts. It was shown that Verbascum species had antimicrobial activity against the Gram (+) bacteria and the yeast, but no activity was seen against the Gram (-) bacteria (Dulger, Kirmizi, Arslan & Guleryuz, 2002).

In this study, antimicrobial activities for the solvent extracts of *C. tomentosa* and *V. mykales*, two endemic plant species from Aydın-Turkey, were examined against some Gram (+) and Gram (-) bacteria, yeasts and microfungi.

MATERIALS AND METHODS

Plant materials

The sample of leaves of *C. tomentosa* was collected from Aydın, Doğanbey village (Turkey) in 2018 (Figure 3a, b) and the leaf sample of *V. mykales* was collected from Aydın, Söke/Samsun Mountain (Turkey) in 2017 (Figure 4a, b). The plants were authenticated by Dr. Özkan EREN. Leaf samples from these plants were collected in an amountsuitable to be used in the study

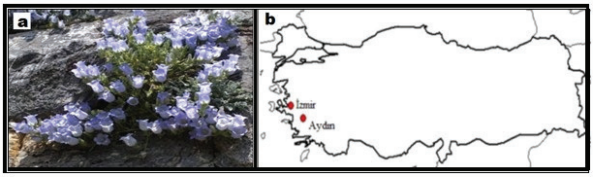

Figure 3. a. *Campanula tomentosa* Lam. (Eren ve Şentürk, 2018) **b.**The geographical distribution of *Campanula tomentosa* Lam. endemic species in Turkey (www.tubives.com).

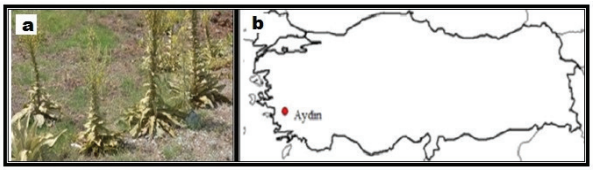

Figure 4. a. *Verbascum mykales* Bornm.(www.turkiyebitkileri.com) **b.**The geographical distribution of *Verbascum mykales* Bornm endemic species in Turkey (www.tubives.com).

without damaging the plant by Dr. Özkan EREN. Both plants are under protection in Turkey. The herbarium numbers of *V. mykales* and *C. tomentosa* are AYDN 2603 and AYDN 2604, respectively.

Preparation of plant extracts

Leaf of the plant samples were washed with distilled water and air-dried. Dried leaf was powdered and 15 grams of the materials were extracted separately in 150 mL of ethyl acetate, methanol, chloroform, acetone and boiled water in a Soxhlet apparatus for 6 h (Göse & Hacıoğlu Doğru, 2021). Then, the extract was filtered and concentrated by rotary evaporator. The dry powder extracts (0.5-1.0g) were kept at +4°C and the extracts were dissolved in 5% DMSO just before the activity studies were started (Törün, Çoban, Bıyık, & Barışık, 2017; Çoban, Bıyık, Törün, &Yaman, 2017).

Microorganisms and condition for cultivation

Seventeen bacteria, three yeasts and three microfungi were used to test the antimicrobial effect. The Gram (-) strains were *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13882, *Pseudomonas aeruginosa* ATCC 35032, *Serratia marcescens* ATCC 13880, and *Proteus vulgaris* ATCC 33420. The Gram (+) strains were *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pneumoniae* ATCC 27336, *Corynebacterium xerosis* ATCC 373, *Mycobacterium smegmatis* ATCC 607, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 19112, *Bacillus cereus* ATCC 11778, and *Bacillus subtilis* ATCC 6633. The yeast strains were *Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Saccharomyces cerevisiae* ATCC 9763, *Aspergillus flavus* ATCC 9807, *Aspergillus niger* ATCC 16404 and *Aspergillus oryzae* ATCC 10124. The strains were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The bacterial strains were cultered in Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA) at 30-37°C for 24 h. The yeast strains were cultured in Malt Extract Agar (MEA) at 30°C for 24 h (Çoban, Erçin, Törün, & Bıyık, 2018; Bıyık, Onur, Törün, & Çoban, 2018). The microfungi strains were cultured in Potato Dextrose Agar (PDA) at 25-27°C for 5-7 days (Okoye, Uba, Dike, & Eziefule, 2020).

Antimicrobial assays

The antimicrobial activities of the two plants were determined by the agar well diffusion method (Collins, Lyne, Grange, & Falkinham, 2004; CLSI, 2004; CLSI, 2015; Balouiri, Sadiki, & Ibnsouda, 2016; EUCAST, 2019). The minimum inhibitory concentrations (MIC) were obtained by the broth dilution method (Jorgensen & Ferraro, 2009; CLSI, 2009; CLSI, 2013). The minimum bacteriocidal concentrations (MBC) and the minimum fungicidal concentrations (MFC) were tested (Zamri, Bakar, Noor, & Fuad, 2020; Stojkovi´c, Dias, Drakuli´c, Barros, Stevanovi´c, Ferreira, & Sokovi´c, 2020).

Disc diffusion method

Screening for antimicrobial activities were carried out by the agar well diffusion method against test microorganisms (Collins, Lyne, Grange, & Falkinham, 2004; CLSI, 2004; CLSI, 2015; EUCAST, 2019). The inoculum suspensions of the tested bacteria and yeasts were prepared from the broth cultures (18-24 h) and the turbidity adjusted using a 0.5 McFarland standard tube to give an equivalent concentration of 1×10^8 bacterial cells/mL, and 1×10^6 yeast cells/mL (Çoban, Erçin, Törün, & Bıyık, 2018; Oyeka, Asegbeloyin, Babahan, Eboma, Okpareke, Lane, Ibezim, Bıyık, Törün, & Izuogu, 2018). The microfungi suspensions were adjusted to 1×10^4 conidia cells/mL (Ismaiel & Tharwat, 2014). In order to test the antimicrobial activity of the plants, 20 mL of Mueller Hinton Agar (MHA) were poured in petri dishes and kept at room temperature to solidify. Then, they were inoculated with strains of bacteria, yeasts and fungi by taking 0.1 mL from the cell culture media. Then, a hole of 6 mm in diameter and depth was made on the top of the agar plates with a sterile stick and was filled with 50 µL of plant extract (1000µg/mL). Then, bacterial cultures were incubated at 30-37°C for 18-24 h, and yeast cultures were incubated at 27-30°C for 18-24 h. The fungi cultures were incubated at 25-27°C for 5-7 days. At the end ofthe incubation time, the diameters of the inhibition zones formed on the MHA were evaluated in mm. Discs containing Chloramphenicol (30 mg Oxoid), Gentamycin (10 mg Oxoid), Tetracycline (30 mg Oxoid), Erythromycin (15mg Oxoid), Penicillin (10 mg Oxoid), Ampicillin (10 mg Oxoid), Vancomycin (30 mg Oxoid), and Ofloxacin (5 mg Oxoid) for bacteria, Nystatin (100 mg Oxoid) for yeasts, and Clotrimazole (10mg Oxoid) for microfungi were used as positive controls. The measured inhibition zones of the extracts were compared with those of the reference discs (Çoban, Bıyık, Törün, &Yaman, 2017).

Dilution method

The antibacterial and antifungal activities of solvent extracts synthesized compounds were examined by preparing a microdilution broth (Jorgensen & Ferraro, 2009; CLSI, 2009; CLSI, 2013). The analysis was carried out in a sterile 96-well microtitre plate. The suspensions, adjusted as 1×10^8 bacterial cells/mL, 1×10^6 yeast cells/mL and 1×10^4 conidia cells/mL for the analysis, were used. Initially, 100 µL of Mueller Hinton Broth (MHB) was placed in each well. After, the extracts were added into the first well. Two-fold serial dilutions of the extracts were carried out to determine the MIC, within the concentration range 256 to 0.25 µg/mL. Next, 100 µL of microorganism suspension was added into each well. The bacterial cultures were incubated at

30-37°C, and yeast cultures were incubated at 27-30°C for 18-24 h. The fungi cultures were incubated at 25-27°C for 5-7 days. The lowest concentration of antimicrobial agent that resulted in complete inhibition of the microorganisms was represented as MIC (µg/mL). Streptomycin for bacteria, and fluconazole for yeasts and microfungi were used as positive controls in the dilution method. In each case, the test was performed in triplicate and the results were expressed as means.

Minimum Bacteriocidal Concentration (MBC) / Minimum Fungicidal Concentration (MFC)

As a result of MIC test was carried out MBC and MFC tests. From each clear well in the MIC assay, 10 µL was inoculated and spread onto MHA plates. Then the plates were incubated at 30-37°C for 18-24 hours for the bacteria, and at 25-27°C for 5-7 days for the fungi. The MBC and MFC were identified as the lowest concentration of extract that did not grow any bacteria and fungi on the MHA plates (Zamri, Bakar, Noor, & Fuad, 2020; Stojkovi´c, Dias, Drakuli´c, Barros, Stevanovi´c, Ferreira, & Sokovi´c, 2020).

Statistical analysis

Mean values and standard deviation calculations were made using SPSSv22 (Statistical Package for Social Sciences).

RESULTS AND DISCUSSION

Antimicrobial analysis

The antimicrobial activity of the ethyl acetate, methanol, chloroform, acetone and boiled water extracts of *C. tomentosa* and *V. mykales* were researched and the results are given Table 1 and 3. Also, the results of the reference antibiotics used are showed in Table 2.

Among the plant extracts tested, the ethyl acetate extracts of *C. tomentosa* and *V. mykales* indicated a high effect against

Table 1. Antimicrobial activities of the extracts of *C. tomentosa* and *V. mykales* against some microorganisms (Inhibition zone mm).

Test Microorganisms	Inhibition zones (mm)									
	Plant Extracts									
	Campanula tomentosa Lam.					*Verbascum mykales* Bornm.				
	EA	C	M	Ac	BW	EA	C	M	Ac	BW
Escherichia coli	17.33±2.51	_	_	_	_	25.33±2.51	_	_	_	_
Enterobacter aerogenes	17.66±2.51	_	_	_	_	19.33±2.08	_	_	_	_
Salmonella typhimurium	15.33±2.51	_	9.66±0.57	_	_	19.66±0.57	_	_	_	_
Micrococcus luteus	21.33±1.52	_	8.66±0.57	_	_	27.33±2.51	_	11.00±1.00	10.66±1.15	
Staphylococcus aureus	13.33±0.57	_	14.33±0.57	_	_	16.00±2.64	_	12.33±2.51	11.66±2.08	_
Staphylococcus epidermidis	17.00±2.64	_	9.33±0.57	_	_	19.33±1.15	_	10.00±0.00	11.66±2.08	_
Klebsiella pneumoniae	19.33±0.57	_	12.66±0.57	_	_	18.00±3.00	_	_	_	_
Streptococcus pneumoniae	15.66±0.57	_	9.33±0.00	_	_	25.00±2.00	_	11.66±2.88		_
Pseudomonas aeruginosa	12.00±1.00	_		_	_	23.00±2.64	9.66±0.57	23.33±2.88	18.66±2.08	_
Corynebacterium xerosis	19.00±2.64	_	12.33±0.57	_	_	19.66±0.57	_	_	_	_
Mycobacterium smegmatis	22.33±1.52	_	10.33±0.57	_	_	19.33±1.15	_	_	_	_
Listeria monocytogenes	19.33±0.57	_	11.00±0.00	10.33±0.57	_	19.00±1.73	_	_	_	_
Serratia marcescens	21.66±2.08	_	8.66±0.57	_	_	21.66±2.88	_	_	_	_
Proteus vulgaris	24.33±1.15	_	11.66±1.15	_	_	20.33±0.57	_	_	_	_
Enterococcus faecalis	23.00±1.00	_	10.66±1.15	_	_	15.00±1.73	_	_	_	_
Bacillus cereus	24.00±1.00	_	14.33±1.15	10.33±0.57	_	19.33±3.21	_	12.00±2.00	12.00±1.73	_
Bacillus subtilis	23.33±1.52	_	11.33±0.57	9.66± 0.57	_	23.00±1.73	_	11.33±1.52	11.66±1.52	_
Candida albicans	13.66±1.15		9.66±1.15	10.33±0.57	_	_	_	_	_	_
Candida utilis	_	_	_	_	_	11.00±1.00	_	_	11.33±2.30	_
Saccharomyces cerevisiae	12.33±1.15		10.00±0.00	_	_	_			_	_
Aspergillus flavus	_	_	_	_	_	12.33±2.51	_	_	_	_
Aspergillus niger	_	_	_	_	_	12.66±0.57	_	_	_	_
Aspergillus oryzae	_	_	_	_	_	_	_	_	_	_

(-): Zone did not occur
EA: Ethyl Acetate, M: Methanol, C: Chloroform, Ac: Acetone, BW: Boiled Water

Table 2. Inhibition zone diameter of the reference antibiotics to test microorganisms (mm).

Test Microorganisms	Inhibition zones (mm)									
	Reference antibiotics									
	C30	CN10	TE30	E15	P10	AMP10	VA30	OFX5	NS100	CTL10
Escherichia coli	24	21	15	11	16	-	23	28	NT	NT
Enterobacter aerogenes	19	20	14	-	-	-	-	19	NT	NT
Salmonella typhimurium	17	16	15	8	15	8	21	25	NT	NT
Micrococcus luteus	25	15	26	30	13	28	14	24	NT	NT
Staphylococcus aureus	23	20	22	23	12	20	13	23	NT	NT
Staphylococcus epidermidis	22	17	19	11	11	17	12	22	NT	NT
Klebsiella pneumoniae	21	19	20	14	18	-	23	27	NT	NT
Pseudomonas aeruginosa	22	20	20	21	14	-	18	29	NT	NT
Corynebacterium xerosis	20	17	25	26	14	27	21	22	NT	NT
Mycobacterium smegmatis	23	18	26	25	16	19	20	30	NT	NT
Listeria monocytogenes	19	14	12	-	10	12	25	29	NT	NT
Serratia marcescens	23	19	13	-	18	19	27	27	NT	NT
Proteus vulgaris	17	24	16	20	15	-	24	26	NT	NT
Enterococcusfaecalis	16	11	19	-	12	14	20	28	NT	NT
Streptococcus pneumoniae	24	20	25	15	19	14	29	28	NT	NT
Bacillus cereus	23	24	25	26	10	-	21	28	NT	NT
Bacillus subtilis	22	20	12	25	11	-	20	27	NT	NT
Candida albicans	NT	NT	NT	NT	NT	NT	NT	NT	22	NT
Candida utilis	NT	NT	NT	NT	NT	NT	NT	NT	21	NT
Saccharomyces cerevisiae	NT	NT	NT	NT	NT	NT	NT	NT	15	NT
Aspergillus flavus	NT	NT	NT	NT	NT	NT	NT	NT	NT	23
Aspergillus niger	NT	NT	NT	NT	NT	NT	NT	NT	NT	24
Aspergillus oryzae	NT	NT	NT	NT	NT	NT	NT	NT	NT	24

(-): Zone did not occur. NT: Not tested
C30: Chloramphenicol (30 mg Oxoid), CN10: Gentamycin (10 mg Oxoid), TE30: Tetracycline (30 mg Oxoid), E15: Erythromycin (15mg Oxoid), AMP10: Ampicillin (10 mg Oxoid), P10: Penicillin (10 mg Oxoid), VA: Vancomycin (30 mg Oxoid), OFX5: Ofloxacin (5 mg Oxoid), NS100: Nystatin (100 mg Oxoid), CTL10: Clotrimazole (10mg Oxoid).

some Gram (-) and Gram (+) bacteria (Table 1). The ethyl acetate extract of *C. tomentosa* showed strong activity (19-24 mm) against *M. luteus*, *K. pneumoniae*, *C. xerosis*, *M. smegmatis*, *L. monocytogenes*, *S. marcescens*, *P. vulgaris*, *E. faecalis*, *B. cereus*, and *B. subtilis*. On the other hand, the ethyl acetate extract of *V. mykales* Bornm. demostrated more powerful effects (18-27 mm) against *E. coli*, *E. aerogenes*, *S. typhimirium*, *M. luteus*, *S. epidermidis*, *K. pneumoniae*, *S. pneumonia*, *P. aeruginosa*, *C. xerosis*, *M. smegmatis*, *L. monocytogenes*, *S. marcescens*, *P. vulgaris*, *B. cereus*, and *B. subtilis* (Figure 5a, b). The ethyl acetate extract of *C. tomentosa* showed significant activity (12-17 mm) against *E. coli*, *E. aerogenes*, S. *typhimirium*, *S. aureus*, *S. epidermidis*, *S. pneumonia*, *P. aeruginosa*, *C. albicans*, and *S. cerevisiae*. The methanole extract of *C. tomentosa* showed a remarkable effect (12-14 mm) against *S. aureus*, *K. pneumoniae*, *C. xerosis*, and *B. cereus*. The same extract and the acetone extract of the plant indicated a slight effect (8-11 mm) against *S. typhimirium*, *M. luteus*, *S. epidermidis*, *S. pneumonia*, *M. smegmatis*, *L. monocytogenes*, *S. marcescens*, *P. vulgaris*, *E. faecalis*, *B. cereus*, *B. subtilis*, *C. albicans*, and *S. cerevisiae*. However, the chloroform and boiled water ex-

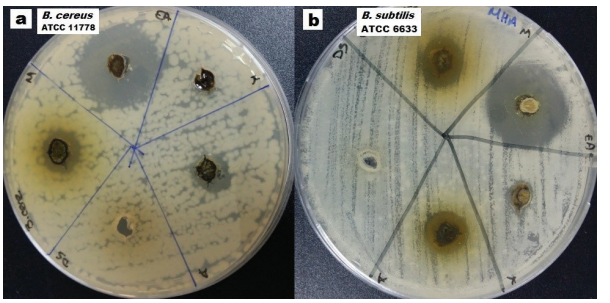

Figure 5. a. Effect of ethyl acetate, methanol, chloroform, acetone, boiled water extracts of *Campanula tomentosa* Lam. against *Bacillus cereus* ATCC 11778 **b.** Effect of ethyl acetate, methanol, chloroform, acetone, boiled water extracts of *Verbascum mykales* Bornm. against *Bacillus subtilis* ATCC 6633. EA: Ethyl Acetate, M:Methanol, K:Chloroform, A:Acetone, DS: Boiled Water.

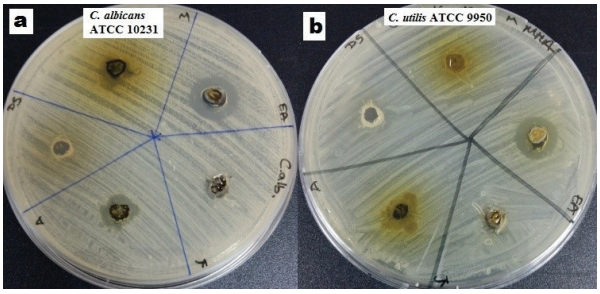

Figure 6. a. Effect of ethyl acetate, methanol, chloroform, acetone, boiled water extracts of *Campanula tomentosa* Lam. against *Candida albicans* ATCC 10231 **b.** Effect of ethyl acetate, methanol, chloroform, acetone, boiled water extracts of *Verbascum mykales* Bornm. against *Candida utilis* ATCC 9950. EA: Ethyl Acetate, M:Methanol, K:Chloroform, A:Acetone, DS: Boiled Water.

tracts of *C. tomentosa* had no effect against *C. albicans* (Figure 6a). The ethyl acetate extract of *V. mykales* Bornm. displayed a noteworthy effect (12-16 mm) against *S. aureus*, *E. faecalis*, *A. flavus*, and *A. niger*. The methanole and acetone extracts of the plant demostrated a high effect (18-23 mm) against *P. aeruginosa* while the same extracts had a moderate effect (12 mm) against *B. cereus*. However, the ethyl acetate and acetone extracts of *V. mykales* Bornm. had a low effect (9-11 mm) against *C. utilis* (Figure 6b). Otherwise, The boiling water extract of the plant has no effect against tested microorganisms.

According to the MIC/MBC/MFC values in Table 3, the ethyl acetate extract of *C. tomentosa* had a strong effect (8 µg/mL) against *M. smegmatis*, *P. vulgaris*, *E. faecalis*, *B. cereus*, and *B. subtilis*. On the other hand, the ethyl acetate extract of the plant showed a significant effect (16-64 µg/ mL) against other bacteria and yeasts. Also, the methanol and acetone extracts of the plant had a very low effect (128-256 µg/mL) against many bacteria and two yeasts. The ethyl acetate extract of *V. mykales* indicated a very strong effect (4-8 µg/mL) against *E. coli*, *M. luteus*, *S. pneumonia*, *P. aeruginosa*, and *B. subtilis*. Besides, the same extract of plant demostrated an appreciable effect (16-64 µg/mL) against other bacteria, one yeast and two microfungi. However, the methanole and acetone extracts of the plant showed a remarkable effect (8-16 µg mL^{-1}) against *P. aeruginosa*. Otherwise, the chloroform, methanole and acetone

Table 3. Antimicrobial activities of the extracts of *C. tomentosa* and *V. mykales* against some microorganisms (MIC/MBC/MFC), (µg/mL).

Test Microorganisms	MIC/MBC/MFC (µg/mL)									
	Campanula tomentosa Lam.				*Verbascum mykales* Bornm.				Reference antibiotics	
	EA	C	M	A	EA	C	M	A	STR	FLK
Escherichia coli	32/64	_	_	_	4/8	_	_	_	64	NT
Enterobacter aerogenes	32/64	_	_	_	16/32	_	_	_	64	NT
Salmonella typhimurium	32/64	_	256/> 256	_	16/32	_	_	_	64	NT
Micrococcus luteus,	16/32	_	256/> 256	_	4/8	_	_	_	32	NT
Staphylococcus aureus	32/64	_	64/128	_	32/64	_	>256/>256	128/ 256	32	NT
Staphylococcus epidermidis	32/64	_	256/> 256	_	16/32	_	>256/>256	128/ 256	32	NT
Klebsiella pneumoniae	16/32	_	64/128	_	16/32	_	_	_	64	NT
Streptococcus pneumoniae	32/64	_	256/> 256	_	4/8	_	>256/>256	_	128	NT
Pseudomonas aeruginosa	64/128	_	_	_	8/16	256/> 256	8/16	16/32	64	NT
Corynebacterium xerosis	16/32	_	64/128	_	16/32	_	_	_	64	NT
Mycobacterium smegmatis	8/16	_	128/ 256	_	16/32	_	_	_	128	NT
Listeria monocytogenes	16/32	_	128/256	256/> 256	16/32	_	_	_	64	NT
Serratia marcescens	16/32	_	256/> 256	_	16/32	_	_	_	64	NT
Proteus vulgaris	8/16	_	128/ 256	_	16/32	_	_	_	64	NT
Enterococcus faecalis	8/16	_	128/ 256	_	32/64	_	_	_	64	NT
Bacillus cereus	8/16	_	64/128	128/ 256	16/32	_	64/128	64/128	64	NT
Bacillus subtilis	8/16	_	128/ 256	256/> 256	8/16	_	128/ 256	128/ 256	64	NT
Candida albicans	64/128	_	256/ >256	128/ 256	_	_	_	_	NT	64
Candida utilis	_	_	_	_	128/ 256	_	_	128/ 256	NT	64
Saccharomyces cerevisiae	64/128	_	128/ 256	_	_	_	_	_	NT	64
Aspergillus flavus	_	_	_	_	64/128	_	_	_	NT	64
Aspergillus niger	_	_	_	_	64/128	_	_	_	NT	64

STR= Streptomycin, FLK= Fluconazole. (-) = No effect, NT: Not tested

extracts had a slight activity (128-256 µg/mL) against some bacteria and one yeast (Table 3).

The antimicrobial effect of methanol, acetone and ethyl acetate extracts obtained from *V. pinnatifidum* Vahl. and *V. antinori* Boiss. et Heldr were researched against Gram (+) and Gram (-) bacteria, and *C. albicans* ATCC 10231. It was found that the *V. antinori* extracts have a greater antimicrobial effect than *V. pinnatifidum* extracts against the test microorganisms (Göse & Hacıoğlu Doğru, 2021). The methanol, dichloromethane, and aqueous crude extracts of *C. retrorsa* flower, leaf and stem were tested against the microorganisms. It was found that the activities of the dichloromethane extracts of leaves and flowers of *C. retrorsa* have a moderate effect against *A. baumanii* and *C. albicans* and the methanol and aqueous crude extracts of *C. retrorsa* have no activity against the other bacteria tested and *C. albicans* (Alhage, Elbitar, Taha, & Benvegnu, 2020). Himalayan medicinal plants were used traditionally to treat pneumonia and tuberculosis. It has been revealed that the methanol extract of *V. thapsus* leaves has antibacterial activity against *S. aureus*, *S. pneumonia* and *M. tuberculosis* (Muhammad, Shandana, Khushboo, & Rahila, 2019). Abdallah & Omar (2019) remarked on the antimicrobial activity of water, ethanol and methanol extracts of the aerial parts of *V. fruticulosum* against an *E. coli* clinical isolate. The results showed that water and methanol extracts did not inhibit *E. coli*; however, the ethanol extract repressed growth of *E. coli*. In another study by Dülger & Dülger (2018), it was reported that the methanol extract obtained from *V. antinori* Boiss. et Heldr. has an antibacterial effect against Gram (+) and Gram (-) bacteria. In asimilar study by Fares (2018), the antimicrobial activity of the methanol, acetone, n-hexane and aqueous extracts of the aerial parts of *V. fruticulosum* were examined against microorganisms. Methanol, acetone and n-hexane extracts of plant has higher activity than the water extract against *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecium*, *S. sonnei*, and methicillin-resistant *S. aureus* (MRSA).The methanol and acetone extracts of plant indicated the strongest inhibition against *P. aeruginosa* (1.56 mg/mL), *E. coli* and *S. aureus* (6.25 mg/mL). The aqueous extract of plant has effect against *S. aureus* (MRSA) (3.125 mg/mL) while the methanol and n-hexane extracts of plant have effect against *E. faecium* and *S. sonnei* (3.125 mg/mL). The methanol, acetone and n-hexane extracts had activity against *C. albicans* and *E. floccosum*. The highest effect (1.56 mg/mL) was obtained with the n-hexane extract against *E. floccosum*. However, the aqueous extract did not have any activity. An antibacterial effect was seen on *S. aureus* ATCC 6538P (22 mm), *B. cereus* ATCC 7064 (20 mm), *L. monocytogenes* ATCC 15313 (14 mm), and *M. luteus* CCM 169 (17mm). The methanol extract of *V. mucronatum* flowers was tested against *E.coli* ATCC 25922, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *C. albicans* ATCC 90028, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 90018. It was found that *V. glabratum subsp. bosnense* (K. Maly) Murb. includes quercetin and rosmarinic acid, 4-hydroxybenzoic acid, salicylic acid, morin, and apigenin as bioactive compounds. In addition, the ethanol extracts of *V. glabratum subsp. bosnense* (K. Maly) Murb. had a moderate effect with MIC values of 600µg/mL – 1200µg/mL against *E. coli, S. aureus,* and *C. albicans* (Karalija, Parić, Dahija, Bešta-Gajević, & Zeljković, 2018). The phenolic profiles and endogenous hormone levels in embryogenic and nonembryogenic calli of *C. tomentosa* were analyzed, but an antimicrobial activity study of this endemic plant species has not been presented in the records (Coşkun, Gemici, &Yildirim, 2017). In another study, the methanolic extracts of *Verbascum cheiranthifolium Boiss. var. asperulum* (Boiss.) Murb. Monorg., *V. pynostachyum* Boiss. & Heldr and *V. orgyale* Boiss. & Heldr.were tested against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. utilis*, *C. glabrata*, and *C. krusei*. It was remarked that *V. pynostactum* and *V. orygale* extracts indicated a higher effect than *V. cheriantifolium var. asperulum*. Particulary, *V. pycnostachyum* extract inhibited *C. krusei* at the concentration of 62.5 µg/mL (Küçük, Özdemir, İşcan, & İncesu, 2016). In a similar study, the antibacterial activity of the methanol and acetone leaf extracts of *V. thapsus* were examined against *E. coli*, *Y. pestis*, *B. cereus*, *P. aeruginosa*, *L. monocytogenes* and *S. aureus*. It was reported that the methanol extract of this plant has stronger activity than an acetone extract against the tested pathogenic bacteria (Prakash, Rana, & Sagar, 2016). The antimicrobial effect of the methanol extract of *V. speciosum* leaves was investigated against *S. aureus*, *L. monocytogenes*, *B. anthracis*, *B. cereus*, *S. typhimurium* and *E. coli*. It was found that the extract has remakable activity against *L. monocytogenes*, *B. cereus*, *S. aureus* and *S. thyphimurium*; even more than penicillin (Nofouzi, Mahmudi, Tahapour, Amini, &Yousefi, 2016). The antifungal activity of a methanol extract of the aerial part of *V. speciosum* was tested against *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. dublinii-ensis*, *A. flavus*, *A. niger*, *Penicillium* sp. and *Alternaria* sp. The highest activity was seen against *C. parapsilosis* and *Alternaria* sp. (Nofouzi, 2015). In another study, the antibacterial activity of some Turkish plants was screened against fish pathogens by Türker & Yıldırım (2015). The ethanol and aqueous crude extracts of *C. glomerata L. subsp. hispida* (Witasek) Hayek and *C. olympica* Boiss. were used for the antibacterial activity. It was reported that the ethanol extracts of *C. glomerata subsp. hispida* and *C. olympica* have a slight effect (11 mm and 8 mm). However, aqueous crude extracts of the plants did not inhibit fish pathogens. In a similar study, the antibacterial effects of ethanol, methanol and water extracts of *C. glomerata* and *C. olympica* flowers, leaves and stems were researched against *S. pyogenes* ATCC 19615, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 13883. It was remarked that the ethanol extract of *C. olympica* has a strong effect (20 mm) against *K. pneumoniae* ATCC 13883 and the extracts of *C. glomerata* and *C. olympica* have high activity against at least one of the tested Gram (-) bacteria. However, the plant extracts had no effect against *S. aureus*, *S. epidermidis* and *P. aeruginosa* (Usta, Yildirim, & Turker, 2014). The antibacterial effect of the aqueous extract of *V. thapsus* was tested against *S. aureus* PTCC1431 and *E. coli* HP101BA 7601c. It was shown that the extract has an effect against Gram (+) and Gram (-) bacteria (Sepahi, Ghorani-Azam, Sepahi, Asoodeh, & Rostami, 2014). In another study, the antimicrobial activity of volatile oil and aqueous extracts of *C. portenschlagiana* Roem.et Schult were evaluated. It was reported that the results of the *C. portenschlagiana* volatile oil have a more powerful antimicrobial activity than the aqueous extract. The volatile oil had very strong activity (19.6-28.3 mm) against Gram (+) and Gram (-) bacteria and its MIC values were 7.8-125.0 mg/mL. However, the aqueous extract of *C. portenschlagiana* indicated considerable effect (10.8-21.5 mm) against the tested bacteria and its MIC values were 125.0-500 mg/mL. The volatile oil of the plant had the most effect (28.3 mm - 7.8 mg/mL) against *P. aeruginosa* FNSST 014 while the aqueous extract of *C. portenschla-*

giana had the most effect (21.5 mm - 125.0 mg/mL) against *S. aureus* ATCC 25923 (Politeo, Skocibusic, Burcu, Maravic, Carev, Ruscic, & Milos, 2013). In another study, the aerial parts of *V. lagurus* Fisch. & C.A.Mey., *V. gnaphalodes* M. Bieb., and *V. xanthophoeniceum* Griseb. were extracted with methanol, chloroform, ethyl acetate and water. The ethyl acetate extract of *V. lagurus* demostrated higher antimicrobial activity among the other *V. lagurus* extracts. The methanol, chloroform, ethyl acetate and water extracts of *V. lagurus* had an effect against *S. aureus* and the MIC values were 156-625mg/L. The methanol extract of *V. lagurus* showed only against *P. aeruginosa*. In addition, the methanol, ethyl acetate and aqueous extracts of *V. lagurus* had a moderate effect against *C. albicans* (Şen, Döşler, & Meriçli, 2012). In a similar study, the antibacterial activity of the aqueous and ethanol extracts of *V. speciosum* flowers were evaluated against *B. subtilis*, *E. aerogenes*, *P. vulgaris* and *S. paratyphi*. It was shown that the ethanol extract had a slight effect (10-11 mm) against *E. aerogenes and S. paratyphi* and the aqueous extract did not inhibit the selected bacteria (Noori, Malayeri, Moosaei, Pakzad, & Piriye, 2012).

When the results of our study are compared with the previous study results, the antimicrobial activities of *C. tomentosa* extracts (especially ethyl acetate and methanol extracts) indicated a higher effect than *C. portenschlagiana*, *C. glomerata*, *C. olympica*, *C. latifolia*, *C. retrorsa*, and *C. lyrata subsp. lyrata* extracts. Also, antimicrobial activities of *V. mykales* extracts were found to be more effective than the antimicrobial activity of other *Verbascum* species.

CONCLUSION

In this study, we investigated the antimicrobial activity of *C. tomentosa* and *V. mykales* endemic plant extracts against some microorganisms. It was determined that ethyl acetate and methanol extracts of plants showed high activity against the tested microorganisms. The results obtained will contribute to the pharmaceutical industry as a novel drug discovery.

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