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Antimicrobial Activity of *Anthemis coelopoda* Var. *bourgaei* Boiss. and *Anthemis tinctoria* Var. *pallida* DC. Species having Ethnobotanical Features

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Abstract: The antimicrobial activity of the ethyl acetate, acetone, chloroform and ethanol extracts from *Anthemis coelopoda* Var. *bourgaei Boiss* and *Anthemis tinctoria* Var. *pallida* DC. were investigated by agar disc diffusion method and all of the extracts exhibited an antimicrobial effect against to most of the tested bacteria. The ethyl acetate extracts of *Anthemis coelopoda* Var. *bourgaei* showed some degree of activity against to some bacteria. Minimal Inhibition Concentration (MIC) of ethyl acetate, ethanol, acetone and chloroform extracts of the samples were determined for some bacteria. In conclusion, MIC value of *A. coelopoda* Var. *bourgaei* against to all of the bacteria tested except for *S. pneumoniae* was lower than the Ciprofloxacin suggesting that it is more effective.

Key words: Antimicrobial activity, *Anthemis coelopoda* Var. *bourgaei*, *Anthemis tinctoria* Var. *pallida*, *ethonobotany*

INTRODUCTION

The genus *Anthemis* (*Asteraceae*) is represented by 62 species in Europe[1]. In Turkey only 50 species have been recorded[2], with a ratio of endemism lying around 54%[3]. These plants prefer dry, open sites on wood-steppe hillsides and grow especially on calcareous substrates in nature. The species of the *Anthemis* genus are widely used in the pharmaceutics, cosmetics and food industry[4,5].

Anthemis tinctoria Var. *pallida* is a 20-45 (-60) cm tall rounded perennial plant, leaves are 2-3 pinnatisect (secondary segments of lower leaves often bearing as many as three pairs of lobes), oblanceolate or obovate in outline. Ligules flowers are white or cream, paleae oblong, acuminate, as long as disc flowers[2].

Anhemis coelopoda Var. *bourgaei* is erect, sparsely pubescent annual. Stems much branched, 20-50 cm. Leaves 2, 5-7 cm, eliptic-oblong in outline, 2-3 pinnatisect into oblong or oblanceolate lobes. Involucre 1-1.5 cm broad; secondary segments of leaves shorter, (2-) 4-5 mm.

For thousands of years, mankind has known about the benefit of drugs from nature.

Plant extracts, for the treatment of various ailments, were highly regarded by the ancient civilizations[6,7]. Of the approximately 250 000 taxa of flowering plants, only 5000 have had their pharmaceutical potential assessed[8]. Plants are still recognized as the bedrock for medicine to treat infectious diseases[9]. Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural antimicrobials[10,11].

This study was conducted to investigate the antimicrobial properties of ethanol, acetone, ethyl acetate and chloroform extracts of *Anthemis coelopoda* Var. *bourgaei* Boiss and *A. tinctoria* Var. *pallida* DC collected from Turkey against to both clinical and food borne microorganisms (bacteria) by using agar disc diffusion method. Minimal Inhibition Concentration (MIC) of ethanol, acetone, ethyl acetate and chloroform extracts of samples were also determined against to some bacteria.

MATERIALS AND METHODS

Plant materials and extraction procedure: Two plant species from *Compositae* in Turkey, were selected on the basis of ethnopharmacological or taxonomic relationship with medicinally important species. Two *Anthemis* taxa were collected from original (typical) localities in Turkey by Celik. *Anthemis coelopoda* Var. *bourgaei Boiss.* were collected from Bilecik-Sogut (June 2003; 1150 m), *A. tinctoria* Var. *pallida* DC. were collected from Bilecik-Sogut (June 2003; 1100 m). The specimens collected were identified with the help of Flora of Turkey and the East Aegean Islands[2] by Uysal and Celik. The

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Table 1: Plant species tested for antibacterial activity

Plant species	Common name	Collector No.	Inhibition zone (mm)	MIC values (μg mL^{-1})	Traditional uses
A. coelopoda Var. *bourgaei*	Papatya	575	13-17^{A} 10-15^{B}	1.9-7.8^{A} 1.9-15.6^{B}	Not known
A. tinctoria Var. *pallida*	Sari papatya Okuz gozu	576	10-12^{A} 13^{D}	7.8-15.6^{A} 7.8^{D}	Dye hair and wool fibre yellow, cancer

A: Ethyl acetate extract, B: Acetone extract, C: Chloroform extract and D: Ethanol extract

Table 2: Antimicrobial activity of *Anthemis coelopoda* Var. *bourgaei* and *Anthemis tinctoria* Var. *pallida* extracts against to the bacterial strains tested based on agar disc diffusion method

	Inhibition zone in diameter (mm/sensitive strains)								
	Anthemis coelopoda Var. *bourgaei*				*Anthemis tinctoria* Var. *pallida*				Standard antibiotic
Microorganisms	A	B	C	D	A	B	C	D	(5 μg)
Escherichia coli	15	10	-	-	10	-	-	-	21
Staphylococcus aureus	15	15	-	-	-	-	-	-	23
Streptococcus pneumoniae	13	-	-	-	10	-	-	-	24
Pseudomonas aeruginosa	17	-	-	-	-	-	-	-	23
Staphylococcus epidermidis	14	-	-	-	10	-	-	-	23
Enterococcus feacalis	14	-	-	-	-	-	-	-	21
Klebsiella pneumonia	14	-	-	-	-	-	-	13	23
Proteus mirabilis	15	-	-	-	12	-	-	-	23
Bacillus cereus	15	11	-	-	10	-	-	-	23
Enterobacter aerogenes	15	-	-	-	10	-	-	-	23

A: Ethyl acetate extract, B: Acetone extract, C: Chloroform extract and D: Ethanol extract,-, Not active

voucher specimens were deposited in the Herbarium of Faculty of Science and Arts, Canakkale Onsekiz Mart University (COMU). Collector numbers were given as Celik (Table 1). The plants previously were air dried and then aerial parts (stem, leaf, flower and fruit) were grinded with the help of Warring Blender. Twenty gram of grinded samples were extracted with 150 mL of ethyl acetate, acetone, chloroform, alcohol solvent (Merck, Darmstadt) for 24 h by using Soxhlet equipment. The extracts were filtered using Whatman filter paper and then concentrated *in vacuum* at 70°C. The residues were stored in a refrigerator until subsequent use.

Microorganisms: The mostly recorded in hospital infection as total 10 bacteria species were used in this study. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pnemoniae* (ATCC 49616), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus feacalis* (ATCC 29212), Klebsiella pneumonia (ATCC 13883), *Proteus mirabilis* (ATCC 7002), *Bacillus cereus* (ATCC 11778) and *Enterobacter aerogenes* (ATCC 13043) were obtained from Anadolu University, Faculty of Science and Arts, Department of Biology, Eskisehir, Turkey.

Antimicrobial activity

Antibacterial test: Antibacterial activity of the extracts was tested against 10 species of Gram-positive and Gram negative bacteria (Table 2). The bacterial cultures used were from the properly identified and stock cultures were obtained from the Anadolu University, Faculty of Science and Arts, Department of Biology, Eskisehir, Turkey.

Agar disc diffusion method: Each microorganism was suspended in sterile saline and diluted at 10^8 colony forming units (cfu) per mL. They were flood-inoculated onto the surface of Mueller Hinton Agar (Oxoid). The discs (6 mm in diameter) were cut from the agar. After incubation for 24 h at 37°C, all plates were determined for any zones of growth inhibition and the diameters of these zones were measured in millimeters. All test were performed in duplicate. Ciprofloxacin (5 μg mL^{-1}) for bacteria were used as standard antibiotics.

Determination of minimum inhibitory concentration: A broth microdilution broth susceptibility assay was used, as recommended by NCCLC, for the determination of the MIC[12]. All the test were performed in Mueller Hinton Broth (Oxoid). Bacterial strains were cultured overnight at 37°C in MHA. The test strains were suspended in MHB to give a final density of 10^8 cfu mL^{-1} and these were confirmed by viable counts. Microplates were incubated at 37°C for 24 h for microorganisms. The bacterial growth was indicated by the presence of a white pellet on the well bottom. Ciprofloxacin was used as standard antibacterial agent.

RESULTS AND DISCUSSION

Traditional uses and common name of the tested plant species and their extracts distribution zone against

Table 3: The MIC of *Anthemis coelopoda* Var. *bourgaei* and *A.tinctoria* Var. *pallida* extracts against to the microorganisms tested in microdilution assay (MIC in μg mL^{-1})

	Anthemis coelopoda Var. *bourgaei*				*Anthemis tinctoria* Var. *pallida*				Standard antibiotic
Microorganisms	A	B	C	D	A	B	C	D	(μg mL^{-1})
E.coli	1.953	15.625	-	-	15.625	-	-	-	≥4
S. aureus	1.953	1.953	-	-	-	-	-	-	≥4
S. pneumoniae	7.813	-	-	-	15.625	-	-	-	≥4
P.aeruginosa	1.953	-	-	-	-	-	-	-	≥4
S. epidermidis	3.906	-	-	-	15.625	-	-	-	≥4
E.feacalis	3.906	-	-	-	-	-	-	-	≥4
K. pneumonia	3.906	-	-	-	-	-	-	7.813	≥4
P. mirabilis	1.953	-	-	-	7.813	-	-	-	≥4
B. cereus	1.953	15.625	-	-	15.625	-	-	-	≥4
E. aerogenes	1.953	-	-	-	15.625	-	-	-	≥4

A: Ethyl acetate extract, B: Acetone extract, C: Chloroform extract and D: Ethanol extract, -, Not active.

to some bacteria are given in Table 1. The antimicrobial activities of *Anthemis coelopoda* Var. *bourgaei*, *Anthemis tinctoria* Var. *pallida* ethanol, acetone, ethyl acetate and chloroform extracts against to microorganisms examined in the present study and their potency were qualitatively assassed by the presence or absence of inhibition zones and zone diameter (Table 2).

The results showed that the ethyl acetate and acetone extracts of *Anthemis coelopoda* Var. *bourgaei* showed some degree of activity against to bacteria but, none of the bacteria showed any inhibition zone against to ethanol and chloroform extracts (Table 2).

The ethyl acetate extract of *Anthemis tinctoria* Var. *pallida* showed some degree of activity against to bacteria but, ethanol extract showed significant antimicrobial activity against to only *K. pneumonia*. None of the bacteria showed any inhibition zone against to acetone and chloroform extracts (Table 2).

The results showed that the ethyl acetate extracts of *Anthemis coelopoda* Var. *bourgaei* showed some degree of activity against to some bacteria (Table 2).

MIC values of the extracts were between 1.953-15.625 μg mL^{-1} as it was observed with the standard antimicrobials (Table 3). MIC value of the ethyl acetate extract *A. coelopoda* Var. *bourgaei* against to all of the tested bacteria except for *S. pneumoniae* was lower (7.813 μg mL^{-1}) than the suggested Ciprofloxacin (≥4 μg mL^{-1}) that it is more effective (Table 3). MIC value of the acetone extract *A. coelopoda* Var. *bourgaei* against to all of the tested bacteria except for *E. coli* and *B. cereus* was lower (15.625 μg mL^{-1}) than the suggested Ciprofloxacin (≥4 μg mL^{-1}) that it is more effective (Table 3).

MIC values of the extracts were between 7.813-15.625 μg mL^{-1} as it was observed with the standard antimicrobials (Table 3). MIC value of the ethyl acetate extract *A. tinctoria* Var. *pallida* against to all of the tested bacteria was higher (7.813-15.625 μg mL^{-1}) than the suggested Ciprofloxacin (≥4 μg mL^{-1}) that it is not effective (Table 3). MIC value of the ethanol extract *A. tinctoria* Var. *pallida* against to all of the tested bacteria except for *K. pneumonia* did not exhibited the significant antimicrobial activities against all the tested bacteria in this study (Table 3). MIC value of the acetone extract *A. tinctoria* Var. *pallida* against to only *K. pneumonia* was higher (7.813 μg mL^{-1}) than the suggested Ciprofloxacin (≥4 μg mL^{-1}) that it is not effective (Table 3).

The results of MIC and agar disc diffusion methods were supported to each other (Table 1).

Based on these results, it is possible to conclude that two *Anthemis* taxa collected from Turkey exhibited with a broad range of antimicrobial activity to varying degrees. Particularly ethyl acetate extracts of *Anthemis coelopoda* Var. *bourgaei* showed significant antibacterial activities which can be used as antimicrobial agents in new drugs for therapy of infectious diseases.

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