Antibacterial Activity of Some Plant Extracts

Nihal Dogruoz[1], Zuhal Zeybek[1*], Ali Karagoz[2]

[1]*Istanbul University, Faculty of Science, Department of Biology, 34134, Vezneciler, Istanbul-Turkey.*
[2]*Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, 34134, Vezneciler, Istanbul-Turkey.*

Abstract

Ten aqueous and one ethanolic extracts from medicinal plants used in Turkey were evaluated for antimicrobial activity. Plant extracts were prepared using distilled water and 50 % ethanol. It was found that three plant extracts from the 9 plants studied had antibacterial activity. These activities were produced by the aqueous extracts of *Pistacia*, *Tilia argentea* and *Anthemis pungens*. All of these plant extracts had antibacterial activity against *E.coli*. Also, *Tilia argentea* and *Pistacia spp* inhibited the growth of B*acillus subtilis*. In addition to these bacteria, *Klebsiella pneumoniae, Staphylococcus aureus* and environmental *Aeromonas spp.* strains were inhibited by *Tilia argentea*.

Keywords: Plant extracts, antibiotics, antibacterial activity, agar well- diffusion.
***Corresponding author:** Zuhal Zeybek (E- mail: zzeybek@yahoo.com)
Abbreviations: AE, aqueous extract; DMSO, dimethyl sulfoxide; EE, ethanolic extract; EMEM, Eagle's minimum essential medium; PBS, phosphate buffer saline; MTT, 3-4,5-dimethylthiazol-2-(yl-2,5-diphenyl tetrazolium bromide; h, hour.

Introduction

Humans have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various maladies. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pub Med during the period between 1966 – 1994, but in the following decade, between 1995 and 2004, 307 were published (Rios and Recio 2005). In a previous study, we have reported the antibacterial effect of lichen extracts against *Legionella pneumophila* strains (Zeybek et al. 2006). The present study describes the evaluation of the antibacterial potency of some plant species from Turkey.

Material and Methods

Collection and identification of plant samples

Plant specimens were collected from Istanbul University Botanical Garden and Kayseri and Silifke regions. Samples were dried at room temperature for 48 h. Their names have been shown in Table 1. *Tilia argentea* and *Anthemis pungens* were classified reference vouchers and deposited at the Herbarium of the Faculty of Science, Istanbul University, (ISTS 38859, 38855).

Preparation of extracts

Two separate samples of the plant material (10 g of each) were air-dried and powdered and were extracted with distilled water (80 mL) and 50% ethanol (75 mL) at room temperature using a waring blender. Plant residues were removed by centrifugation (15000 rpm, 30 min, 4^{o}C) and the supernatant was filtered and evaporated to dryness under reduced pressure and/or lyophilized. In this way, two different crude extracts were obtained: aqueous extract (AE), 50% ethanolic extract (EE). AE was dissolved in the medium Phosphate buffer

saline (PBS) and EE was dissolved in dimethyl sulfoxide (DMSO).

Cell culture

Vero cells were grown and maintained in Eagle's minimum essential medium (EMEM) with Earle's saline, supplemented with an antibiotic-antimycotic mixture [penicillin (100 U/mL), streptomycin (100 µg/mL), amphotericin B (0,25 µg/mL)] and 10% fetal calf serum. Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C.

Cytotoxicity assay

The cytotoxicity assays were performed according to the microculture 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (Quintero et al. 1999; Mantanıi et al. 2001; Mosmann 1983; Kawase et al., 2003). The cells were harvested (4,5 – 5 x 10^4 cells/well) and inoculated in 24 well microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the extract. The final concentration of DMSO did not exceed 0.2% (v/v), a concentration without effect on cell replication. After 72 h incubation, the medium is aspirated. 150 µL of MTT solution (5 mg/mL in PBS, pH 7.2) is added to each well and the plates incubated for 4 h at 37°C. After incubation, 800 µL of DMSO was added to each well of plates, followed by gentle shaking to solubilize the formazan dye for 15 min. Absorbance was read at 540 nm using a spectrophotometer and surviving fraction calculated.

Bacterial strains

In this study, the bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 4352, *Staphylococcus aureus* ATCC 6538, and *Staphylococcus epidermidis* ATCC 12228) , two environmental *Aeromonas* spp. (*Aeromonas* spp 1, *Aeromonas* spp 2) strains, that are isolated from different lakes, were used.

Antimicrobial Activity Determination

Agar-well diffusion was used in the antimicrobial activity tests. Mueller Hinton Agar was used as media.

Agar Well Diffusion

The bacteria suspensions were prepared equal to the turbulence of Mac Farland 0.5 standart and were cultivated (100 µl) on agar medium. After that 6 mm diameter were punched in agar plate. The standart antibacterial agent cephalothin and tobramycin (30 µg/ml) were tested in the same manner as control. All the plates were incubated at 37°C, for 18-24 hours. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed (NCCLS 1997; Ulusoylu et al. 2001). Each test was performed twice and the average of the results was taken. The solvent (ethanol 50 %) was not affected the growth of any of the bacteria. The standart antibacterial agents (cephalothin and tobramycin) were carried out all tested bacteria.

Results

The result of screening plant extracts for antimicrobial activity was summarized in Table 2. The cytotoxic effect of each extract was examined with the cell viability of Vero cells. It was found that some plant extracts had cytotoxic activity in different degrees. The concentrations selected for antimicrobial activity tests were noncytotoxic concentration (Table 1).

Antibacterial activities were produced to different extents by the aqueous extracts of *Pistacia*, *Tilia argentea*, *Anthemis pungens*. Although *Tilia argentea* inhibited the growth of five bacteria, the other two plants showed antibacterial activity against *E. coli*, *B. subtilis* and *S. aureus* (Table 2). Also, all of the plant extracts had antibacterial activity against *E.coli*.

It was seen that the inhibition zone of bacteria by plant extracts have changed between 10-18 mm. In comparison with reference antibiotics cephalothin and tobramycin was found 15 – 40 mm except *P. aeruginosa* (Table 2).

Table 1. Plants used in this study and the concentration of plant extracts used in experiments.

Plants	Concentration
Digitalis purpurea L.	5 μg/mL (H_2O)
Digitalis purpurea L.	5 μg/mL (ETOH)
Cardiac glikozid from *Digitalis purpurea*	1 μg/mL (H_2O)
Sanicula europaea L.	100 μg/mL (H_2O)
Anthemis pungens.	250 μg/mL (H_2O)
Ecballium elaterium L.	1 μg/mL (H_2O)
Urtica dioica L.	250 μg/mL (H_2O)
Nerium oleander L.	1 μg/mL (H_2O)
Tilia argentea.	250 μg/mL (H_2O)
Juglans regia,	100 μg/mL (H_2O)
Pistacia sp.	100 μg/mL (H_2O)

Discussion

The result of screening plant extracts for antimicrobial activity is summarized in Table 2. The noncytotoxic concentrations of plant extracts were used for antimicrobial activity tests (Table 1). Our findings suggest that the antimicrobial activity is not due to the cytotoxic activity of extracts. Antibacterial activities were produced to different extents by the aqueous extracts of *Pistacia*, *Tilia argentea* and *Anthemis pungens*.

In this study, it was found that the inhibition zones of plant extracts were smaller than those of antibiotics (Table 2). Although all three plants which had antibacterial effect on *E.coli* bacteria, their effects changed against other tested bacteria (Table 2). All plants were examined totally and all experiments were done with only their water extracts. It is known that a lot of factors effect antibacterial activity. So, we think that the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the organism tested. In fact, there are a lot of different researches from our country. Arıkan (1992) that showed that although fruit extracts of some seed plants had antibacterial activity against *E.coli*, they had not this effect against some other bacteria. Also, in that study was reported that fruit and leaf extracts of some seed plants had antibacterial effect against *Bacillus megaterium, Pseudomonas aeruginosa, Staphylococcus aureus* but their leaf extracts had not this effect against *E.coli*. Dülger et al. (1998) found that although four different extracts of a Macrofungus had antibacterial activity against *Bacillus subtilis* ATCC 663 and other some Gram positive and Gram negative bacteria, they had not this activity against *E.coli* ATCC 11230, *S. epidermidis* NRRL B- 4377, *S. aureus* ATCC 6538P. Uğur et al. (1996) showed water extracts of *Tribulus terrestris* had not antimicrobial effect against *E.coli* ATCC 8739, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633, *K.*

Table 2. Antimicrobial effect of plant extracts

SAMPLES	BACTERIA Inhibition zone (mm)							
	E.coli	*P. aeruginosa*	*B. subtilis*	*K. pneumoniae*	*S. aureus*	*S. epidermidis*	*Aeromonas spp (1)*	*Aeromonas spp (2)*
***Digitalis purpurea* L**	-	-	-	-	-	-	-	-
***Digitalis purpurea* L.**	-	-	-	-	-	-	-	-
Cardiac glikozid from *Digitalis purpurea*	-	-	-	-	-	-	-	-
***Sanicula europaea* L.**	-	-	-	-	-	-	-	-
***Anthemis pungens*.**	**12**	-	-	-	**16**	-	-	-
***Ecballium elaterium* L.**	-	-	-	-	-	-	-	-
***Urtica dioica* L.**	-	-	-	-	-	-	-	-
***Nerium oleander* L.**	-	-	-	-	-	-	-	-
Tilia argentea	**13**	-	**10**	**10**	**18**	-	-	**11**
Juglans regia	-	-	-	-	-	-	-	-
Pistacia sp.	**12**	-	**12**	-	-	-	-	-
Tobramycin	**15**	**22**	**17**	**20**	**15**	**18**	**20**	**20**
Cephalothin	**21**	**0**	**22**	**15**	**40**	**20**	**28**	**34**
H_2O	-	-	-	-	-	-	-	-
EtOH	-	-	-	-	-	-	-	-

pneumoniae ATCC 4352 and some other bacteria and yeasts. Gücin et al. (1997) reported that lichen *Pseudevernia furfuracea* (L.) Zopf had revealed antimicrobial activities on some Gram positive bacteria and yeasts.

In addition to these topical studies, it could also seen in similar studies in world literature. It has been determined that lichens have showed antibacterial activity against *Bacillus, Pseudomonas, E.coli, Streptococcus, Staphylococcus, Enterococcus, Mycobacterium* (Esimone and Adikwn 1999; Ingólfsdóttir K. 2002; Perry et al. 1999). Behera et al. (2005) reported that the acetone, methanol, and light petroleum extracts of lichen *Usnea ghattensis* were effective against *B. licheniformis, B. megaterium, B. subtilis* and *S. aureus*. Yam et al. (1997) showed that aqueous extracts of teas (*Camellia sinensis*) of different types and from various sources inhibited a wide range of pathogenic bacteria, including methicillin-resistant *S. aureus*.

Consequently, the antibacterial activity of plants tested can be explained with new studies by using different solvents for extraction, other bacteria and different parts of plants, accurately.

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