

Full Length Research Paper

Antimicrobial activity of some endemic plant species from Turkey

Mehlika Benli^{1*}, Kerim Güney², Ümit Bingöl¹, Fatmagül Geven¹ and Nazife Yiğit³

¹Ankara University, Faculty of Science, Department of Biology, Ankara, Turkey.

²Kastamonu University, Faculty of Forestry, Kastamonu, Turkey.

³Kirikkale University, Faculty of Science and Arts, Department of Biology, Kirikkale, Turkey.

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Six plant extracts obtained from different parts such as the leaves, flowers and seeds of four species of the endemic plants in Turkey were tested on a total of 14 microorganisms, 10 of which were bacterial strains and 4 yeast strains. *Verbascum eriocarpum* (flower) extract was found to be effective against *Staphylococcus aureus*; *Stachys cretica* subsp. *anatolica* (leaf and flower) and *Heracleum paphlagonicum* (seed) extracts were found to be effective against *Bacillus subtilis*; and *Alcea apterocarpa* (seed and sepal) extract was found to be effective against *Pseudomonas aeruginosa*. No antimicrobial activity was observed in *Heracleum paphlagonicum* (leaf) and *Alcea apterocarpa* (leaf) plant extracts. The minimum inhibitory concentration (MIC) values of the plant extracts were calculated to be between ≥ 0.859 mg/ml and ≥ 110.5 mg/ml and the minimum bacteriocidal concentration (MBC) values were calculated to be between ≥ 3.44 mg/ml and ≥ 132 mg/ml.

Key words: Antimicrobial activity, endemic plants, plant extract.

INTRODUCTION

Turkey has a considerably notable and rich flora in terms of existing plant diversity. It is located in a region where three fitogeographical regions intersect and constitutes a bridge between Southern Europe and Southwestern Asia and Anatolia. Therefore, it is the origination and diversification centre of many genus and sections and it has ecological and fitogeographical diversity. As a consequence, endemism of species is high (Tan, 1992; Dağcı et al., 2002).

Traditional medicines have been used for a wide variety of purposes for many thousands of years in Turkey and all over world. In particular, extracts and oils of these plants have formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicine, and natural therapies. However, most of the applications have not been evaluated scientifically, and their effects have not been explained

experimentally yet. About 25% of the medicines used for people's health have been obtained from these plants so far (Şener et al., 1996). Recently, the antimicrobial activity of various plant extracts against many microorganisms has been studied in Turkey (Baytop, 1984; Ertürk, 2006; Keleş et al., 2001; Dıđrak et al., 2002; Dölger, 2005; Dölger and Gonuz, 2004).

Up to now an increasing number of antibiotic-resistant bacteria have been reported (Rawat and Uniyal, 2003; Dölger et al., 1999; Parvathi and Brindha, 2003) and thus new natural therapeutic agents are needed in order to eradicate these pathogens. Through the discovery of plants that have antimicrobial activity, it will be possible to discover new natural drugs serving as chemotherapeutic agents for treatment of nosocomial pathogens and take these antibiotic-resistant bacteria under control.

The aim of this paper was to investigate the antimicrobial effects of six plant extracts obtained from different parts of four endemic plants such as *Verbascum eriocarpum* (flower), *Stachys cretica* subsp. *Anatolica* (leaf and flower), *Heracleum paphlagonicum* (leaf), *H. paphlagonicum* (seed), *Alcea apterocarpa* (leaf), and *A. apterocar-*

*Corresponding author. E-mail: mehlikabenli_76@hotmail.com.
Phone: +90 312 212 67 20 – 1505. Fax: +90 312 223 23 95.

pa (seed and sepal).

MATERIALS AND METHODS

Collection of plants

From the A4, Kastamonu: Azdavay; Karyatağı Mountain, Yanık Plateau, 41°41.05N - 33°20.25E, 1141 m, 15.08.2006, Endemic, Iran-Turan, KG 1493 region, *V. eriocarpum* (Freyn. and Sint.) Bornm., *S. cretica* L. subsp. *anatolica* Rech., *H. paphlagonicum* Czecczott., *A. apterocarpa* (Fenal) Boiss. Plants were collected and dried in the shade. These plants were identified at Ankara University Faculty of Science Herbarium (ANK Herbarium). The plant samples are being preserved at Ankara University Faculty of Science Herbarium (ANK Herbarium).

Preparation of extracts

For the determination of antimicrobial activity, 3 g of ground plant parts were soaked in 30 ml of methanol, and for the minimal inhibitory concentration (MIC) tests, 10 g of ground plants were extracted with 100 ml of methanol for 24 h. Then, the extracts were filtered and dried. The dry weight of the remaining residue was calculated. The dried material was again re-dissolved in methanol and diluted with deionized water. After the prepared extract solution was sterilized at 121°C for 15 min, it was placed in sterile tubes and centrifuged at 5000 rpm for 3 min. The supernatant of extract was used.

Test microorganisms

Fresh cultures of the microorganisms were grown in Nutrient broth (Acumedia). The density of microorganisms was adjusted to McFarland 0.5 standard. In the tests, a total of 14 microorganisms including; *Enterococcus gallinarum* CDC-NJ-4, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* RSHI, *Escherichia coli* RSHI, *Shigella* RSHI, *E. coli* ATCC 25922, *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC27853, *Saccharomyces cerevisiae* (Pakmaya), *Candida albicans* 845981, *Candida crusei* ATCC 6258 and *C. albicans* 900628 were used. For negative control, 1 ml of methanol – 5 ml of deionized water mixture was used, and for positive control amikacin (30 µg/ml) (Eczacibasi), vancomycin (30 µg/ml) (Mayne), penicillin (10U/ml) (I.E.Ulagay), gentamicin (10 µg/disc) (I.E.Ulagay), rifamicin (5 µg/ml) (Aventis), tetracycline (30 µg/ml) (SIGMA), ampicillin (10 µg/ml) (SELVA), chloramphenicol (30 µg/ml) (SIGMA) and erythromycin (15 µg/ml) (SIGMA) antibiotics were used. In the MIC tests, gentamicin (Genta-120 mg) (I.E.Ulagay) was used as the standard antibiotic.

Determination of antimicrobial activity

Fresh cultures of the microorganisms (100 µl) were inoculated on Muller Hinton Agar (Merck). Agar was allowed to dry for 15 - 20 min and on each plate, three drops each of which was 20 µl of the extract was dropped. The plates were then incubated at 37°C for 24 h and the diameters of inhibition zones were measured and evaluated. The assays that were found to be effective were repeated three times. The positive and negative tests were performed by using the same conditions (Bilgehan, 2004).

Determination of minimum inhibitory concentration (MIC)

On the sensitive bacterial strains, two-fold liquid dilution tests were made by using Mueller Hinton Broth (Merck). For each strain, two

series of 10 tubes were used; while in the first series the plant extract was tested, in the second series standard antibiotic was tested. Whether bacterial growth occurred or not was determined by observing the turbidity of the cultures. The tube in which no growth occurred was evaluated as the minimum inhibitory concentration (MIC) and then the minimum effect dose of the extract was calculated (Bilgehan, 2004).

Determination of the minimum bacteriocidal concentration (MBC)

The contents of the MIC tubes having no-growth were spread on Mueller Hinton Agar plates for colony counting. MBC was calculated by the determination of whether the activity of the extract was bacteriostatic or bacteriocidal according to the state of growth. If there was no growth, the extract was identified as bacteriocidal (Bilgehan, 2004).

RESULTS AND DISCUSSION

In our study, Karyatağı Mountain region in Kastamonu, Turkey was selected as the research field. The locality of the region is at 16 - 18° North latitudes and 27 - 31° East longitudes. Azdavay is located in its southern part, Şenpazar is located in its northern part, Ağlı in its eastern part and Devrekani creek in its western part. The vertical limits of the research field are between 873–1210 m. Six extracts obtained from different parts of four plants from among the plants collected from this region and included in the endemic flora were tested on 10 bacterial and 4 yeast strains.

It was observed that *V. eriocarpum* (flower) extract was effective against *S. aureus*; *S. cretica* subsp. *Anatolica* (leaf and flower) and *H. paphlagonicum* (seed) extracts were effective against *B. subtilis*; and *A. apterocarpa* (seed and sepal) extract was effective against *P. aeruginosa*. No antimicrobial activity was observed in *H. paphlagonicum* (leaf) and *A. apterocarpa* (leaf) extracts (Table 1).

When the antimicrobial activities of standard antibiotics and plant extracts were compared, it was shown that plant extracts have effects that were similar to those of antibiotics and moreover, *A. apterocarpa* (seed and sepal) extract had an inhibitory effect against *P. aeruginosa* more than the antibiotics do. Even though this bacterium was resistant against the majority of the antibiotics tested, it was found to be susceptible to the plant extract and exhibited an inhibition zone of 36 mm.

The content concentrations of the plant extracts tested can clearly be seen in Table 2. Again in Table 2 it can be seen that the MIC values of the plant extracts were between ≥ 0.859 mg/ml and ≥ 110.5 mg/ml. MBC values were found to be between ≥ 3.44 mg/ml and ≥ 132 mg/ml. Among the plant extracts, the antimicrobial activities of *H. paphlagonicum* (seed) extract was the one with the minimum effective dose and at the same time the most effective since even a content concentration of 0.859 mg/ml was sufficient to eradicate the bacteria. In the

Table 1. The antimicrobial activity of six extract that were extracted four endemic plants different parts and nine standard antibiotics

MICROORGANISMS	INHIBITION ZONES (mm)															
	PLANT EXTRACTS						C	STANDARD ANTIBIOTICS								
	<i>Verbascum eriocarpum</i> (flower)	<i>Stachys cretica subsp. anatolica</i> (leaf and flower)	<i>Heracleum paphlagonicum</i> (leaf)	<i>Heracleum paphlagonicum</i> (seed)	<i>Alcea apterocarpa</i> (leaf)	<i>Alcea apterocarpa</i> (seed and sepal)	Negative control	Amikacin	Vancomycin	Penicillin	Gentamicin	Rifocin	Tetracycline	Ampicilin	Chloramphenicol	Erythromycin
<i>Enterococcus gallinarum</i> CDC-NJ-4	-	-	-	-	-	-	-	-	12	-	15	13	-	-	-	11
<i>Enterococcus faecalis</i> ATCC 29212	-	-	-	-	-	-	-	16	12	-	16	14	-	-	-	11
<i>Bacillus subtilis</i> RSHI	-	20	-	11	-	-	-	24	19	22	25	23	12	-	13	24
<i>Escherichia coli</i> RSHI	-	-	-	-	-	-	-	18	-	-	18	-	-	-	-	-
<i>Shigella</i> RSHI	-	-	-	-	-	-	-	20	-	-	19	-	-	-	-	-
<i>Escherichia coli</i> ATCC 25922	-	-	-	-	-	-	-	16	-	-	17	-	-	-	-	-
<i>Streptococcus pyogenes</i> ATCC 19615	-	-	-	-	-	-	-	13	12	-	16	15	-	-	-	12
<i>Staphylococcus aureus</i> ATCC 29213	12	-	-	-	-	-	-	17	15	19	17	27	12	-	-	18
<i>Listeria monocytogenes</i> ATCC 7644	-	-	-	-	-	-	-	25	16	-	27	39	-	-	-	19
<i>Pseudomonas aeruginosa</i> ATCC27853	-	-	-	-	-	36	-	17	-	-	15	-	-	-	-	-
<i>Saccharomyces cerevisiae</i> (Pakmaya)	-	-	-	-	-	-	-	-	16	-	-	17	10	8	-	11
<i>Candida albicans</i> 845981	-	-	-	-	-	-	-	17	12	-	19	15	-	-	-	12
<i>Candida crusei</i> ATCC 6258	-	-	-	-	-	-	-	14	11	-	17	17	-	-	-	11
<i>Candida albicans</i> 900628	-	-	-	-	-	-	-	17	11	-	16	15	-	-	-	11

Table 2. MIC, MBC values of the *Crataegus tanacetifolia* leaf extract (mg/ml) and Gentamicin ($\mu\text{g/ml}$) on susceptible bacterial strains.

Plant extracts	Bacteria	Extract Concentration (mg/ml)	Gentamicin ($\mu\text{g/ml}$)	MIC (mg/ml)	MBC (mg/ml)
<i>Stachys cretica</i> subsp. <i>Anatolica</i> (leaf and flower)	<i>B. subtilis</i>	187	≥ 1.875	≥ 46.75	≥ 46.75
<i>Heracleum paphlagonicum</i> (seed)	<i>B. subtilis</i>	220	≥ 1.875	≥ 0.859	≥ 3.44
<i>Verbascum eriocarpum</i> (flower)	<i>S. aureus</i>	264	< 1.875	≥ 66	≥ 132
<i>Alcea apterocarpa</i> (seed and sepal)	<i>P. aeruginosa</i>	221	≥ 1.875	≥ 110.5	≥ 110.5

MBC tests, no bacterium colony was observed in the inoculations made on agar plates. These results indicate that the plant extracts have a bacteriocidal effect on bacteria. The effect of the plant extract on bacterial strains was not in a manner of blocking growth but eradicating pathogenous bacteria.

Many antimicrobial activity studies have been performed on different plant species belonging to *Heracleum* genus. In these studies, it is reported that they were effective on many bacterial and yeast strains (Fazly and Haririzadeh, 2003; Shahidi Bonjar et al., 2004; Dash et al., 2005; Kuar et al., 2006; Ergene et al., 2006). Similarly, the antimicrobial activities of extracts obtained from different parts and different species of *Verbascum* (Dülger et al., 2002; Guarino, 2002; Dulger and Gonuz, 2004; Şengül et al., 2005) and *Stachys* (Diğrak et al., 2001; Dulger and Gonuz, 2004) were shown.

These results indicate that different parts of same plant species have different antimicrobial effects. Even if extracts are prepared using the same parts (leaves or fruits or seeds) of the same plant species, it is possible that the bacteria on which they are effective and the degrees of the effect may vary. Factors such as the structure of soil, daily and seasonal changes during the collection of the plant material, and the physiological growth cycle of the plant may cause variations in the chemical compounds of the plant. In addition to these factors, the parts of plants, extraction process, solvent, and the species of bacteria that are used are also variables (Izzo et al., 1995; Martínez et al., 1996).

Consequently, some of these plant extracts have antibacterial activity, and the activities eradicate the bacteria completely (bacteriocidal effect). Effective compounds to be obtained by the determination of the active compound in the plant can provide new resources for chemotherapeutics to be synthesized. It will be a base to our further investigations on advanced purification.

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