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Full length Research paper

An examination on the antimicrobial activity of some prevalent plant variety from Turkey.

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In this investigation performed on six prevalent plant variety, antimicrobial action was seen in Campanula lyrata subsp.lyrata and Abies nordmanniana subsp. bornmuelleriana plants. The base inhibitory grouping of C. lyrata subsp. lyrata (leaf and bloom) separate was observed to be ≥ 30 mg/ml for Baccillus subtilis and ≥ 15.5 mg/ml for Staphylococcus aureus, and the base inhibitory focus (MIC) of Abies nordmanniana subsp. bornmuelleriana (leaf) remove was observed to be > 314 mg/ml for B. subtilis and when least bacteriocidal fixation (MBC) comes about were assessed, it was watched that the plant removes had bacteriocidal impacts. No antimicrobial movement was seen in the other plant separates, to be specific, Onosma bornmuelleri (leaf-bloom), Dianthus balansae (leaf-blossom), Alyssum pateri subsp. pateri (seed) and Scabiosa columbaria subsp. paphlagonica (leaf) removes that were tried.

Key words: Antimicrobial action, prevalent plants, plant variety, Scabiosa columbaria, seed.

INTRODUCTION

As in all the countries in the world, the use of plants for medical treatment purposes in our country goes back to very old times. According to an investigation conducted by World Health Organization (WHO) in 91 countries, the total number of medical plants used for treatment purposes is around 20,000. It is reported that from among these, around 500 plants are being grown. Besides, very few of the plants used for various purposes are registered in the pharmacopies (Codex). For instance, the number of plants registered in the Turkish codex is around 140; whereas the number of plants used for medicinal purposes among the people is quite large (Kirbag, 1999).

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

Today, people all over the world are trying to keep away from chronic stress, pollution and synthetic drugs (Perumalsamy et al., 1998). Keeping synthetic drugs away from the nature and human body is extremely difficult, their supply is hard and/or expensive and above all, microorganisms that are resistant against these synthetic agents arise day by day and an increase in their number is observed. All these negativities have brought natural agents to the fore and have brought alternative and complementary medicine up to date (Dulger et al., 1999, Rawat and Uniyal, 2003). The common view in the society and scientific community is that the natural agents are healthier, harmless and more reliable when compared to synthetic products (Parvathi and Brindha, 2003).

Many scientific data-based studies on the roots, stems, leaves, seeds, flowers and fruits of many plants having antimicrobial characteristics have been performed and the antimicrobial activity results of plants have been reported (Leven et al., 1979, Erturk and Demirbag, 2003,

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Sudharameshwari and Radhika, 2007).

In our study, the antimicrobial activity of *Campanula lyrata subsp.lyrata* (Campanulaceae), *Onosma bornmuelleri* (Boraginaceae), *Dianthus balansae* (Caryophyllaceae), *Abies nordmanniana subsp. bornmuelleriana* (Pinaceae), *Alyssum pateri subsp. pateri* (Brassicaceae), *Scabiosa columbaria subsp. paphlagonica* (Dipsacaceae) which are some endemic plant species were determined. These plants are included in the endemic flora of the region and were collected from the research field, Karyatagi Mountain (Kastamonu / Azdavay). The extracts that were prepared using different parts were tested on ten bacterial and four yeast strains in terms of antimicrobial activity.

MATERIALS AND METHOD

Collection of plants

Endemic six plant species were collected from Karyatagi Mountain, Yanik Plateau, Kastamonu city in Turkey in August 2006. The locality of the region is at 16 - 18° North latitudes and 27 - 31° East longitudes and Azdavay is located in its southern part, Senpazar is located in its northern part, Agli in its eastern part and Devrekani creek in its western part. The vertical limits of the research field are between 873 -1210 m.

The plants were dried in the shade. These six plants were *C. lyrata subsp. lyrata* (leaf and flower), *O. bornmuelleri* (leaf and flower), *D. balansae* (leaf and flower), *A. nordmanniana subsp. bornmuelleriana* (leaf), *A. pateri subsp. pateri* (seed) *S. columbaria subsp. paphlagonica* (leaf).

The plants were identified at Gazi University Faculty of Science Herbarium (ANK Herbarium), and their leaves, seeds and flowers were used in tests. The plant samples are being preserved at A Gazi University Faculty of Science Herbarium (ANK Herbarium).

Test microorganisms

Fresh cultures of the microorganisms which were developed in Nutrient broth (acumedia) were used. The density of microorganisms was adjusted as per Mc Farland 0.5 standard. In the tests; a total of 14 microorganisms being *Enterococcus gallinarum* CDC-NJ-4, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* RSHI, *Escherichia coli* RSHI, *Shigella* RSHI, *Escherichia 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 *Candida albicans* 900628 were used. These microorganisms were obtained from Microbiology Laboratory Culture Collection of Refik Saydam Hifzisihha Institute (RSHI).

Preparation of plant extract

For antimicrobial activity tests, 3 g were soaked in 30 ml of methanol and for the minimal inhibitory concentration (MIC) tests, 10 g of ground plants were soaked in 100 ml of methanol and it was gently heated. Then, it was kept over night. At the end of the night, the extracts were filtered and kept in an incubator at 45ºC until the methanol evaporated. The dry weight of the remaining residue was measured. The dry material obtained was redissolved in 3 ml of methanol and diluted with deionized water.

Determination of antimicrobial activity

The extracts were diluted at a rate of 1/5 and tested on microorga-nisms using the drop method. The density of the microorganisms was adjusted as per McFarland 0.5 Standard and the micro-organisms were grown in Nutrient broth (acumedia). On each of the Muller Hinton Agar (Merck) plates on which microorganisms were inoculated (100 µl), three drops of sterile extract solution of were dropped (20 µl). The plates were then incubated at 37ºC for 24 h and the diameters of inhibition zones that were formed were measured and evaluated. The assays that were found to be effective were repeated three times. The positive and negative tests were performed using the same method. In the drop method, while 1 ml of methanol and 5 ml of deionized water mixture was used as negative control; amikacin (30 µg/ml) (Eczacibasi), vancomycin (30 µg/ml) (Mayne), penicillin (10 U/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) standard antibiotics were used as positive control. In the MIC tests, gentamicin (Genta-120 mg) (I.E.Ulagay) was used as the standard antibiotic (Bilgehan, 2004).

Determination of minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC)

These tests were performed on the bacteria that exhibited inhibition zones using the effective extracts. The extracts that exhibited considerable activity were diluted double fold (2:2) with Muller Hinton Broth (Merck) in a series of ten test tubes. An aliquot of 0.5 ml of the bacterial suspension (Mc Farland 0.5) was used. The same process was repeated using Gentamicin (Genta 120 mg – Đ.E. ULUGAY) and it was used as positive control. All tubes were incubated at 37^{o}C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. MIC results were given as the result of incubation of 24 h and the results of incubations of 18, 24, 48 and 72 h were also evaluated. The contents of all tubes that showed no visible growth were cultured

Table 1. The effects of six plant extracts and nine standard antibiotics on microorganisms and the means of zone diameters they have exhibited (mm).

MICROORGANISMS	INHIBITION ZONES (mm)															
	PLANT EXTRACTS						C	STANDARD ANTIBIOTICS								
	Campanula lyrata subsp. lyrata(leafandflower)	*Onosma bornmuelleri*(leafandflower)	*Dianthus balansae*(leafandflower)	*Abies nordmanniana subsp.bornmuelleriana*(leaf)	*Alyssum pateri subsp. pateri*(seed)	*Scabiosa columbaria subsp.paphlagonica*(leaf)	Negative control	Amikacin	Vancomycin	Penicillin	Gentamicin	Rifocin	Tetracycline	Ampicilin	Chloramphenicol	Erythnomycin
Enterococcus gallinarum CDC-NJ-4	-	-	-	-	-	-	-	-	12	-	15	13	-	-	-	11
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	16	12	-	16	14	-	-	-	11
Bacillus subtilis RSHI*	14	-	-	14	-	-	-	24	19	22	25	23	12	-	13	24
Escherichia coli RSHI*	-	-	-	-	-	-	-	18	-	-	18	-	-	-	-	-
Shigella RSHI*	-	-	-	-	-	-	-	20	-	-	19	-	-	-	-	-
Escherichia coli ATCC 25922	-	-	-	-	-	-	-	16	-	-	17	-	-	-	-	-
Streptococcus pyogenes ATCC 19615	-	-	-	-	-	-	-	13	12	-	16	15	-	-	-	12
Staphylococcus aureus ATCC	27	-	-	-	-	-	-	17	15	19	17	27	12	-	-	18

29213																
Listeria monocytogenes ATCC 7644	-	-	-	-	-	-	-	25	16	-	27	39	-	-	-	19
Pseudomonas aeruginosa ATCC27853	-	-	-	-	-	-	-	17	-	-	15	-	-	-	-	-
Saccharomyces cerevisiae (Pakmaya)	-	-	-	-	-	-	-	-	16	-	-	17	10	8	-	11
Candida albicans 845981	-	-	-	-	-	-	-	17	12	-	19	15	-	-	-	12
Candida crusei ATCC 6258	-	-	-	-	-	-	-	14	11	-	17	17	-	-	-	11
Candida albicans 900628								17	11	-	16	15	-	-	-	11

RSHI: Microbiology Laboratory Culture Collection of Refik Saydam Hifzisihha Institute. (-): No inhibition zone (resistant).

Table 2. MIC extract dilution rates of the bacteria that are susceptible to the plant extract at the 18th, 24th, 48th and 72nd h.

Plant extracts	Bacteria	MIC 18 h	MIC 24 h	MIC 48 - 72 h
Campanula lyrata subsp. lyrata	*B. subtilis*	1/8	1/8	1/2
Campanula lyrata subsp. lyrata	*S. aureus*	1/32	1/16	1/2
Abies nordmanniana subsp. bornmuelleriana	*B. subtilis*	(+)	(+)	1/64

Table 3. MIC, MBC concentration values (mg / ml) of the *Campanula lyrata subsp.lyrata* and *Abies nordmanniana subsp. bornmuelleriana* extract on susceptible bacterial strains and MIC concentration values of the standard antibiotic Gentamicin on the same bacteria (µg/ml).

Plant extract	Bacteria	Extract conc. (mg/ml)	Gentamicin (µg/ml)	MIC (mg/ml)	MBC (mg/ml)
Campanula lyrata subsp. lyrata	*B. subtilis*	232	≥ 1.875	≥ 29	≥ 116
Campanula lyrata subsp. lyrata	*S. aureus*	232	< 1.875	≥ 14.5	≥ 116
Abies nordmanniana subsp. bornmuelleriana	*B. subtilis*	314	≥ 1.875	> 314	≥ 4.91

on Muller Hinton Agar, incubated at 37^{o}C for 24 h. The minimum bacteriocidal concentration was considered as the lowest concentration that could not produce a single bacterial colony (Bilgehan, 2004).

RESULTS AND DISCUSSION

Karyatagi Mountain region in Turkey, Kastamonu was selected as the research field. *C. lyrata* subsp. *lyrata* (leaf and flower), *O. bornmuelleri* (leaf and flower), *D. balansae* (leaf and flower), *A. nordmanniana* subsp. *bornmuelleriana* (leaf), *A. pateri* subsp. *pateri* (seed) *S. columbaria subsp. paphlagonica* (leaf) which are included in the endemic flora were collected from this region and the extracts obtained from them were tested on microorganisms. *C. lyrata* subsp. *lyrata* extract and *A. nordmanniana subsp. bornmuelleriana* extract were found to be effective on some bacteria. No activity was observed in the other plant extracts (Table 1). *C. lyrata subsp.lyrata* extract was effective against *B. subtilis* and *S. aureus* strain and *A. nordmanniana subsp. bornmuelleriana* extract was found to be effective against only *B. subtilis* strain.

In the minimum inhibitory concentration tests, at the end of incubation periods of 18, 24, 48 and 72 h, turbidity was observed in different tubes that contained extracts of different dilutions (Table 2). The main stock concentration of *C. lyrata subsp.lyrata* extract was calculated as 232 mg/ml and the main stock concentration of *A. nordmanniana subsp. bornmuelleriana* extract was calculated as 314 mg/ml. The minimum inhibitory concentration of *C. lyrata subsp.lyrata* extract was calculated as ≥ 29 mg/ml for *B. subtilis* and ≥ 14.5 mg/ml for *S. aureus*, and the minimum inhibitory concentration of *A. nordmanniana subsp. bornmuelleriana* extract was calculated as > 314 mg/ml for *B. subtilis* (Table 3).

The minimum bacteriocidal concentration of *C. lyrata subsp.lyrata* extract was calculated as ≥ 116 mg/ml for *B. subtilis* and *S. aureus*, and the minimum bacteriocidal concentration of *A. nordmanniana subsp. Bornmuelleriana* extract was calculated as ≥ 4.91 mg/ml for *B. subtilis* (Table 3).

As in many countries (Fazly Bazzaz and Haririzadeh, 2003; Duraipandiyan et al., 2006), numerous studies have been performed in Turkey (Keles et al., 2001; Dulger et al., 2002; Dulger and Gonuz, 2004; Dulger, 2005) on the antimicrobial activity of endemic plant species. It was found out that when different species and different parts of those plants were studied, they exhibited antimicrobial activity. For instance, it was found out that while *O. bornmuelleri* leaf and flower extract was inactive in our studies, *O. argenta-tum* and *O. hispidum* seed extracts and *O. bulbotrichum* plant extract had antibacterial activity (Naz et al., 2006; Ozgen et al., 2003; Fazly Bazzaz and Haririzadeh, 2003). Again, while no activity was observed in *D. balansae* (leaf and flower) extract in our studies, it was shown that the leaf extracts of *D. coryophyllum* which is another species of *Dianthus* had antibacterial activity against the microorganisms tested (Erturk, 2006; Shahidi Bonjar, 2004: a, b, c).

In this study, *C. lyrata subsp. lyrata* plant species was proved to have antimicrobial activity on two bacterial strains from among the microorganisms tested and no similar researches and findings were found with respect to this plant species and other *Campanula* species. No antimicrobial research on *A. nordmanniana subsp. bornmuelleriana* was found but it has been reported that different species and parts of *Abies* have antimicrobial effect. The essential oil of *A. balsamea* was found to be inactive against *E. coli* and active against *S. aureus* with an MIC of 56 µg/ml (Pichette et al., 2006). Again, in another study, the seed lipids of *A. nordmanniana* were found to be most effective against the tested microorga-nisms (Digrak et al., 2002).

In the studies performed by use of dry leaf extracts of different species of *Abies*, the methanol extract of leaves of *A. webbiana* showed a broad spectrum antimicrobial activity (Vishnoi et al., 2007). On the other hand the methanol extract of the leaves of *A. cilicia* was found to be active against *B. subtilis* and *S. aureus* (Digrak et al., 1999).

All these studies indicate that plants have potential antimicrobial activity and even if plants of the species are collected from different regions, they exhibit different activities. Because of both the differences in species and the differences in the parts that are extracted, it is natural that there are differences in their antimicrobial activities. The important thing is the determination of the antimicro-bial activities that plants have and their usability in the preparation of new drugs. Consequently, from among the plant extracts tested in our study, *C. lyrata subsp. lyrata* and *A. nordmanniana subsp. bornmuelleriana* plant extracts have antibacterial activity.

This activity eradi-cates the bacteria completely (bacteriocidal effect). Effective compounds to be obtained by the determination of the active compound in the plant can account for new resources for chemotherapeutics to be synthesized. Even at a trace level, presence of antibacterial active agents in the plant will allow for the preparation of new drugs with new biological agents as a result of obtaining that active agent from the plant through different methods and purification process.

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