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SCREENING OF SELECTED PLANTS GROWING IN IRAN FOR ANTIMICROBIAL ACTIVITY*

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Abstract – The antimicrobial activity of methanolic extracts from different parts of 11 indigenous wild plant species used in traditional medicines of Iran were tested against nine species of microorganisms: *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Bacillus subtilis, Aspergillus niger* and *Candida albicans*. The antimicrobial efficacy was determined using the disk diffusion (0.5, 1, 2 and 4 mg/disk) and minimal inhibition concentration (MIC) method. Among the 11 tested herbs, 9 plants showed antimicrobial activity against one or more species of microorganism. The most active antimicrobial plants were *Stachys obtusicrena, Anvillea garcinii, Salvia* species, *Otostegia persica* and *Teucrium persicum*.

Keywords – Antimicrobial activity, methanolic extract, Iran

1. INTRODUCTION

According to an investigation conducted by the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [1]. Systematic screening of folk medicine plants may result in the discovery of novel effective compounds. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants [2]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials. It may also have a significant clinical value in the treatment of resistant microbial strains [3].

Several studies have been published concerning many properties such as antibacterial, antitumor, antifungal, and antioxidant activity in some species in these genera [4-8]

In this study, methanolic extracts of aerial parts of 11 plants were screened for their antimicrobial activity (Table 1). The plants in this investigation were gathered from different parts of Fars Province. Some of them are commonly used by rural inhabitants as herbal medicines.

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2. MATERIALS AND METHODS

a) Collection of plant material

The aerial parts of eleven indigenous plant species of three families (Astraceae, Apiaceae and Labiatae) were collected from Fars Province, in the south of Iran from May 2004 until June 2006. The taxonomic identification of plant species were confirmed by Dr. Khosravi in the Department of Biology, Shiraz University, Shiraz, Iran. A voucher specimen has been deposited in the herbarium of Shiraz University, Shiraz, Iran.

b) Preparation of the extracts

The aerial parts of the plant species were dried at room temperature, and powdered. The methanol extracts were obtained by maceration of the crude plant powder with methanol/water (90/10) for 2 days at room temperature. The extracts were then filtered using sterile cloth sheets and dried under reduced pressure at temperature below 45˚C.

c) Tested microorganisms

The plant extracts were screened against eight bacterial strains and one fungal strain. The bacteria used were *Escherichia coli* (PTCC 1338), *Bacillus subtilis* (PTCC 1023), *Staphylococcous aureus* (PTCC 1112), *Staphylococcus epidermis* (PTCC 1114), *Pseudomonas aeruginosa* (PTCC 1074), *Salmonella typhi* (PTCC 1693), *Klebsiella pneumonia* (PTCC 1031), *Aspergillus niger* (PTCC 5010) and one fungal strain *Candida albicans* (PTCC 5027) obtained from the Persian type culture collection (PTCC), Tehran, Iran.

d) Antimicrobial activity

The antimicrobial effects were tested by the disc-diffusion method [9] and the minimum inhibitory concentration (MIC) by serial dilution tube method [10].

e) Disk diffusion method

In each investigation, microorganisms were cultured at 37˚C for 16-24 h and prepared at a turbidity equivalent to McFarland standard No. 0.5 [10].

The suspensions were then spread on the test plate (Muller-Hinton agar). Sterile discs were impregnated with 0.5, 1, 2 and 4 mg of the plant extracts and placed on the surface of the test plate. Positive control discs were gentamicin for Gram negative bacteria, ampicillin for Gram positive bacteria and Ketoconazole for *Candida albicans*. Each extract and control was tested in duplicate and the experiment was repeated four times.

f) Minimum inhibitory concentration (MIC) method

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion (Kirby-Bauer) method. The highest dilution of a plant extract that still retains an inhibitory effect against the growth of a microorganism is known as MIC [11]. The complete protocol of the MIC test is found in the M7-T2 publication of the National Committee for Clinical Laboratory Standards [12]. Briefly, each selected plant extract was subjected to a serial dilution using sterile nutrient broth medium as a diluent. The plant extract volumes to broth medium volume (v/v) were 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. Each plant extract dilution was inoculated with 20 µl of an individual microorganism present in its log phase. All inoculated dilutions were set at 37˚C for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism

is recorded as the MIC value of the extract. It is worth noting that the nutrient broth diluent used in dilutions of 1:2, 1:2.5, 1:3, and 1:3.5 was a double strength, while that used for preparing the 1:4 dilution was a single strength. A control experiment was run in parallel to study the impact of the solvent itself (without plant components) on growth of the nine test organisms. Each solvent (water or methanol) was diluted in a similar pattern with sterile nutrient broth, as indicated above, and inoculation by microorganisms followed by incubation were done similarly.

3. RESULTS AND DISCUSSION

The results of the antimicrobial screening of the methanol extracts of all species plants are shown in Tables 1 and 2. Among the plants screened, *Salvia* species, *Stachys obtusicrena, Anvillea garcini, Otoestgia persica,* and *Teucrium persicum* showed good activity against the tested microorganisms. As it can be seen, the best effect of *Anvillea garcini, Teucrium persicum, Salvia santolinifolia, Salvia mirzayanii* and *Stachys obtusicrena* was against gram negative bacteria (*E. coli and P. auruginosa*), the greatest effect of *Salvia santolinifolia, Salvia eremophila* and *Otoestgia persica* was against gram positive bacteria (*S. aureus* and *B. subtilis*) and *Teucrium persicum* and *Anvillea garcinii* showed a good activity against *Candida albicans.*

Several studies on the antimicrobial activity of the essential oils of many Salvia species have been carried out, demonstrating that among the constituents of the oil, some of them showed antimicrobial activity [5, 6]. But the present investigation, however, represents the screening of antimicrobial activity of methanolic extracts. Moreover, former studies on *Otostegia persica* extract showed antimicrobial activity against gram positive [8], which correlates with the observation of the present study.

Table 1. Antimicrobial activity of the methanolic extracts of collected plants

Plant name	Conc.(mg/disk) methanolic extract	Zone of inhibition (mm)				
		Bs	*Sa*	*Ec*	*Pa*	*Ca*
Teucrium persicum	0.5	6.4	6.4	6.4	6.4	6.5
	1	6.6	6.7	6.4	6.4	7.0
	2	6.6	7.0	6.7	7.0	7.5
	4	6.8	7.3	7.7	7.7	8.2
Salvia mirzayanii	0.5	6.8	6.4	6.4	6.4	6.4
	1	8.7	7.1	7.7	6.7	7.0
	2	9.5	10.5	8.0	8.2	8.7
	4	14.5	12.2	11.7	9.5	9.5
Salvia santolinifolia	0.5	6.4	7.7	7.7	6.4	7.0
	1	7.0	10.1	8.7	6.7	8.7
	2	7.7	10.5	11.7	8.2	10.2
	4	9.2	12.2	13.2	9.5	12.7
Salvia reuterana	0.5	6.4	6.7	6.4	6.4	6.4
	1	7.0	7.0	6.7	6.7	6.7
	2	7.7	7.5	7.0	7.0	8.0
	4	8.7	8.0	8.0	9.0	10.0

Table 1. (Continued)

Plant name	Conc.(mg/disk) methanolic extract	Zone of inhibition (mm)				
Salvia macrosiphon	0.5	6.4	7.3	6.4	6.4	6.4
	1	7.3	7.7	7.5	6.8	6.4
	2	8.7	10.5	9.0	7.5	7.0
	4	10.0	14.2	11.7	9.0	7.5
Salvia eremophila	0.5	7.3	6.4	6.7	6.4	6.4
	1	7.7	7.3	7.7	7.7	6.7
	2	10.5	8.7	10.0	8.7	7.2
	4	14.2	10.0	11.5	10.2	8.7
Stachys obtusicrena	0.5	6.6	6.6	6.4	6.5	6.4
	1	6.7	7.0	6.4	6.8	6.5
	2	7.5	7.7	6.4	8.2	7.2
	4	9.7	9.2	6.6	11.7	8.5
Francoeuria undulata	0.5	6.4	6.4	6.4	7.0	6.4
	1	6.4	6.4	6.4	7.7	7.0
	2	7.2	8.0	7.5	9.2	8.0
	4	7.3	11.7	9.0	12.5	8.2
Ducrosia anethifolia	0.5	6.4	6.4	6.4	6.4	6.4
	1	6.4	7.0	7.5	6.7	6.4
	2	6.7	7.5	8.5	7.7	6.4
	4	7.7	8.0	10.7	8.7	6.7
Anvillea garcinii	0.5	6.7	6.8	7.2	6.7	6.7
	1	7.7	7.5	8.0	7.7	7.7
	2	9.0	9.7	9.7	9.0	8.5
	4	11.7	13.0	15.0	11.5	9.2
Otostegia persica	0.5	6.7	6.8	7.2	6.4	6.4
	1	7.7	7.3	8.0	6.7	6.7
	2	9.0	7.5	9.7	7.7	7.1
	4	11.7	9.7	15.0	9.0	7.7
Ampicilin		25.0	35.0			
Gentamicin				15.0	15.0	
Ketoconazol						10.0

Zone of inhibition including the diameter of filter paper disc (6mm)

Ampicillin 10 μg/disc was used as reference for gram positive bacteria Gentamicin 10 μg/disc was used as reference for gram positive bacteria Ketoconazole 10 μg/disc was used as reference for fungus. Bs–*Bacillus subtilis*; Sa–*Staphylococcus aureus*; Ec–*Escherichia coli*; Pa–*Pseudomonas aeruginosa*; Ca–*Candida albicans*

Table 2. Minimum inhibitory concentration (MIC) of collected plants

Plant name	MIC (mg/cc)								
	Es	*Se*	*Kp*	*St*	*Pa*	*Sa*	*Bs*	*Ca*	*An*
Salvia mirzayanii	1	1	1	1	0.25	1	1	0.5	1
Salvia macrosiphon	0.5	1	1	1	1	1	1	0.5	0.12
Salvia santolinifolia	0.25	1	0.5	2	1	1	2	1	1
Salvia eremophylla	2	2	2	1	0.5	1	1	2	0.25
Stachys obtusicrena	0.5	0.25	0.5	0.5	0.5	0.5	1	1	1
Anvillea garcinii	0.25	2	0.5	1	0.5	2	2	0.5	1
Teucrium persicum	0.5	2	2	2	1	1	2	0.5	0.5
Otostegia persica	1	1	0.5	1	1	2	0.5	1	0.5
Francoeuria undulata	4	4	4	4	2	4	4	2	2

Ec–*Escherichia coli*; Se–*Staphylococcus epidermis*; Kp–*Klebsiella pneumonia*; St–*Salmonella typhi*; Pa–*Pseudomonas aeruginosa*; Sa–*Staphylococcus aureus*; Bs–*Bacillus subtilis*; Ca–*Candida albicans*; An–*Aspergillus niger*

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