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Antimicrobial Activity of Extracts *Diplotaenia damavandica* from Iran

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Abstract: *Diplotaenia damavandica* is a perennial wild herb which grows exclusively in central Alborz Mountains, Damavand, Iran. Various extracts of *Diplotaenia damavandica* prepared by methanol, water, n-Hexan and ethyl acetate were studied against bacterial and fungal standard strains using the disk diffusion method and minimum inhibitory concentration (MIC) assay. Extracts of root and leaf exhibited varying degree of antimicrobial activity. All the extracts showed antibacterial activity while methanol, ethylacetate and n-hexan extracts showed antifungal properties.

Key words: Antimicrobial · Diplotaenia damavandica · Extract

INTRODUCTION

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years. These purposes vary from the use of rosewood and cedarwood in perfumery, to flavoring drinks with lime, fennel or juniper berry oil and the application of lemongrass oil for the preservation of stored food crops [1]. Also, plant secondary metabolites, such as essential oils and plant extracts are studied for their antimicrobial activities and most essential oils and extracts derived from plants are known to possess insecticidal, antifungal, antibacterial and cytotoxic activities [2]. Because of the increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial. Therefore, they are intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology and food preservation.

Parsley family (Apiaceae) is well represented in the Iranian flora with at least 112 genera and 316 species of which 75 are endemic [3]. *Diplotaenia damavandica* Mozaffarian, Hedge & Lamond is a perennial wild herb which grows exclusively in central Alborz Mountains around Tar Lake, Damavand, Iran. The plant is locally called kozal and upon contact with skin followed by exposure to sunlight causes photosensitization. Extracts obtained from the aerial parts of the plant have been reported to have antifungal activity and contain furanocumarins [4].

The aim of this study was to test various extracts from leaf and root of *D. damavandica* plant against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and yeasts to evaluate its antibacterial and fungal activities.

MATERIALS AND METHODS

Plant Material: Leaves and root of *D. damavandica* were collected from the Tar Lake area, Damavand, Iran, at an altitude of 2200 m around June - September 2006. A voucher specimen (No.: 85055) is deposited at the Herbarium of Biology Department, Shahid Beheshti University.

Microbial Strains: The antimicrobial activity of *D. damavandica* various extracts individually tested against a panel of microorganisms, including; *Bacillus subtilis* (ATCC 465), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus*

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epidermidis (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 85327), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763). Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA). Yeast was cultured overnight at 30°C in Sabouraud dextrose agar (SDA).

Preparation of Plant Extract: The hot water extract (w) was obtained by maceration of the air-dried and powdered plant material with hot water for 30 min and then held for 24 h at room temperature. The extracts was filtered and held below 5°C. A portion of dried plant material (10g) was extracted by maceration with ethyl acetate (E), methanol (M) and n-hexane (H) for 24 h at room temperature.

Antimicrobial Screening by the Disk Diffusion Method: The disc diffusion method was applied for the determination of antimicrobial activities of the prepared extracts [5]. Extracts were dissolved in dimethyl sulfoxide (DMSO). A suspension of the tested microorganism (0.1 ml of 10^8 cells/ml) was spread over the surface of agar plates (MHA and SDA). Filter papers having a diameter of 6 mm, soaked with 25 µL of each extracts were placed on the inoculated agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 2 h. Then they were incubated at 37 °C for 24 h for bacteria and at 30°C for 48 h for the yeast. The diameters of the inhibition zones were measured in millimeters.

Minimum Inhibitory Concentrations (MIC): MIC values were determined by broth microdilution assay recommended by the NCCLS [6]. Serial two-fold dilutions of the extracts were made in Mueller-Hinton Broth containing 0.5% Tween 80 for bacteria and Sabouraud Dextrose Broth with 0.5% Tween 80 for fungi in 96-well microtiter plates. Fresh microbial suspensions prepared from overnight grown cultures in the same media were added to give a final concentration of 5×10^5 organisms/ml. Controls of medium with microorganisms or the extract alone were included. The plates were incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for fungi. The first dilution with no microbial growth was recorded as MIC.

Statistics: The statistical analysis was performed with statgraphics (Centurion XV) and Excel software. The multi-factorial ANOVA analysis followed by the Tukey multiple comparison tests were used for statistical comparisons. P-value of less than 0.05 was assumed for significant differences.

RESULTS AND DISCUSSION

As can be seen in Table 1 and 2, all extracts were found to have moderate to high activity against *B. subtilis*, *S. epidermidis* and *S.aureus*. *P. aeruginosa* was resistant in all experiments. All extract inhibited slightly the growth of *K.pneumoniae*, *E.coli* and *E.faecalis*. Results obtained from disc diffusion method, followed by measurements of MIC, indicated that *S.epidermidis* was the most sensitive microorganisms tested, to the methanol extract of the plant leaf as it showed the lowest MIC value (1.87 mg/ml).

In general, Gram positive bacteria seemed to be more sensitive to the extracts than gram negative bacteria. The extracts with the strongest antibacterial action are also active on fungi. However, treatment must be continued over a longer period. Leaf extracts showed better antifungal properties than root extracts

Table 1: Antimicrobial activity of various extracts of *D. damavandica* by disc diffusion method

	Leaf extracts (IZ)				Root extracts (IZ)				Antibiotic (IZ)		
Microorganisms	M	W	E	H	M	W	E	H	Tet	Gen	Nys
B. subtilis	20±0.4	18±0.6	18±0.7	24±0.5	15±0.7	17±0.4	11±0.9	24±0.4	21±0.7	0	nt
E. faecalis	11±0.6	0	12±0.4	12±0.8	0	0	12±0.7	0	9±0.9	0	Nt
S. aureus	18±0.7	17±0.7	19±0.9	16±0.6	12±0.4	11±0.6	17±0.5	17±0.5	20±0.6	0	Nt
S. epidermidi	25±0.4	18±0.9	19±0.5	22±0.9	17±0.6	18±0.9	23±0.4	25±0.4	34±0.5	0	Nt
E. coli	12±0.9	11±0.4	13±0.8	14±0.4	10±0.5	12±0.7	12±0.6	14±0.7	0	23±0.4	Nt
P. aeruginosa	0	0	0	0	0	0	0	0	0	12±0.6	Nt
C. albcians	13±0.9	0	19±0.8	18±0.4	9±0.8	0	13±0.4	11±0.9	Nt	Nt	18±0.7
S. cerevisiae	17±0.4	0	18±0.9	20±0.6	11±0.9	0	10±0.8	13±0.6	nt	nt	18±0.9
K. pneumoniae	11±0.7	0	11±0.6	9±0.7	0	0	14±0.8	12±0.4	0	20±0.9	Nt

Valeus given as mean of triplicate tests. M, W, E and H represent Methanol, Water, Ethyl acetate and n-Hexane extracts, respectively. IZ: Inhibition zone (20 µl per disc). Tet: Teracycline, tested at 30 µg/disc.Gen, Gentamicin, tested at 10 µg/disc. Nys: nystatine, tested at 30 µg/disc. Nt = not tested.

Table 2: Antimicrobial activity of various extracts of *D. damavandica* by MIC value.

	Leaf extracts				Root extracts				Antibiotic		
Microorganisms	M	W	E	H	M	W	E	H	Tet	Gen	Nys
B. subtilis	3.75±0.9	3.75±0.7	7.5±0.9	3.75±0.4	7.5±0.8	7.5±0.9	15±0.9	3.75±0.8	3. 2±0.7	nt	nt
E. faecalis	15±0.7	>15	15±0.8	15±0.5	nt	nt	15±0.5	nt	6. 4±0.5	nt	nt
S. aureus	3.75±0.5	7.5±0.9	7.5±0.4	7.5±0.7	15±0.5	15±0.4	7.5±0.7	7.5±0.9	3. 2±0.9	nt	nt
S. epidermidis	1.87±0.8	3.75±0.5	7.5±0.5	3.75±0.9	15±0.7	7.5±0.8	3.75±0.4	3.75±0.5	1. 6±0.8	nt	nt
E. coli	15±0.9	15±0.8	15±0.7	7.5±0.8	>15	15±0.9	15±0.6	15±0.6	nt	3. 2±0.7	nt
K. pneumoniae	15±0.5	>15	15±0.9	>15	nt	>15	15±0.9	15±0.7	nt	3. 2±0.9	nt
P. aeruginosa	>15	nt	nt	nt	nt	nt	15±0.7	10±0.4	nt	6.4±0.5	nt
C. albcians	15±0.7	nt	3.75±0.4	7.5±0.6	>15	nt	15±0.8	15±0.5	nt	nt	3. 2±0.8
S. cerevisiae	3.75±0.9	nt	5.87±0.6	7.5±0.7	15±0.9	nt	15±0.6	15±0.8	nt	nt	1. 6±0.7

Valeus given as mean of triplicate tests. M, W, E and H represent Methanol, Water, Ethyl acetate and n-Hexane extracts, respectively. MIC: Minimum inhibitory concentration values in mg/ml. Tet: Teracycline, tested at 30 µg/disc.Gen, Gentamicin, tested at 10 µg/disc. Nys: nystatine, tested at 30 µg/disc. Nt = not tested.

against *C.albicans* and *S. cerevisiae* . The standard drugs used namely, nystatin, tetracycline and gentamaicin exhibited antimicrobial activities nearly the same as the leaf extracts.

The fact that *E. coli* ATCC 25922 is not inhibited by these extracts is surprising, because this strain is normally very sensitive to antimicrobial agents. The reason for this behavior is not readily apparent and it seems likely that no adequate explanation will be available until the purified "active" components can be tested against these organisms [7].

Antimicrobial activity of extract of other plant species on our tested bacteria or fungi or other types of microorganisms have been previously studied [8-10] and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel were showed antimicrobial activity. Extract such as sweet almond, carrot and mandarin were shown to possess little or no antimicrobial activity.

In our previous study [4], antimicrobial activities of essential oils from leaf, seed and root were studied and results showed that all essential oils showed antibacterial activity by the disk diffusion assay. However, the best results were obtained with the leaf oil which was active not only against the bacterial strains but also produced good zones of inhibition against the fungal test organisms.

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