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SCREENING OF DESERT PLANTS FOR USE AGAINST BACTERIAL PATHOGENS IN FISH

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Key words: antibacterial activity, desert plants, fish, pathogenic bacteria

Abstract

The antibacterial activity of aqueous extracts of 104 desert plant species was studied on the following fish bacterial pathogens: *Aeromonas hydrophila*, *Photobacterium damsela* subspecies *piscicida*, *Streptococcus iniae*, and *Vibrio alginolyticus*. Seventeen plant species had antibacterial activity, as identified by disk diffusion assay. The pathogen *P. damsela* was sensitive to all 17 active extracts except *Peganum harmala* and a high inhibitory effect (14-19.5 mm) was produced by *Anchusa strigosa*, *Hammada scoparia*, *Achillea fragrantissima*, *Pulicaria crispa* and *Loranthus acaciae*. The pathogens *A. hydrophila* and *V. alginolyticus* were inhibited by *H. scoparia*, *L. acaciae*, and *P. harmala* (7-20.5 mm). The pathogen *S. iniae* was inhibited by *Ochradenus baccatus* and *Reseda stenostachya* (10.5 mm). The benefits of using desert plants as an alternative to conventional antibiotics are discussed.

Introduction

A major issue in intensive fish culture is bacterial disease. In an attempt to keep production undisrupted, synthetic antibiotics are often relied upon for disease control. However, continuous use of synthetic antibiotics poses threats to consumers and the environment. Thus, treatment with plants and/or plant parts

that have antibacterial activity is a potentially beneficial alternative. While organic agriculture has developed alternative methods and treatments to combat plant diseases, information on use of natural medicines to treat fish diseases has been limited. Antibiotics derived from phytochemicals may be toxic, but since

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these are natural, their decomposition would be faster.

Plants have been used to treat infectious diseases throughout the history of mankind. Secondary metabolites play a role in the medicinal properties of plants (Briskin, 2000). It may be that long exposure to extreme environmental conditions results in the development of unique secondary metabolites that confer adaptive advantages to plants in extreme environments (Croteau et al., 2000). Therefore, we hypothesized that desert plants may contain secondary metabolites with antibacterial activity that can be used to treat bacterial diseases in fish.

The Negev desert flora includes about 600 species of which 104 were available from the desert plant collection in the laboratory of Prof. Golan at the Jacob Blaustein Institute of Desert Research of the Ben Gurion University of the Negev (Table 1; Sathiyamoorthy et al. 1997a,b, 1999). The bacteria tested in this

study are commonly occurring pathogens in aquaculture (Plumb, 1999).

Materials and Methods

Bacteria. Bacteria were aseptically isolated from diseased fish from commercial aquaculture farms in Israel. *Aeromonas hydrophila* was isolated from African catfish (*Clarias gariepinus*, case no. RV-03-01), *Streptococcus iniae* from tilapia (*Oreochromis* sp., case no. MO-05-01), and *Vibrio alginolyticus* from angelfish (*Pterophyllum scalare*, case no. SH-05-01) on brain heart infusion agar (BHI-A) at the Aquatic Animal Health Laboratory of the Jacob Blaustein Institute of Desert Research. *Photobacterium damsela* subspecies piscicida (previously *Pasteurella piscicida*) from hybrid striped bass (*Morone* sp., case no. 2315) was obtained from Dr. Shula Nitzan at the Fish Health Laboratory at Kibbutz Nir David. *A. hydrophila*, *S. iniae*, and *V. alginolyticus* were identified using the Biolog

Table 1. 104 plant species used in the study.

Family and plant species	Collection site	Plant parts ^a
AIZOACEAE		
<i>Aizoon canariense</i>	DM	whole plant
<i>Mesembryanthemum nodiflorum</i>	Ein Mor	stems, leaves, flowers
	Zin	stems, leaves
AMARANTHACEAE		
<i>Aerva javanica</i>	DM	whole plant
APIACEAE		
<i>Pituranthos tortuosus</i>	DM	bulbs
	Andartat Hanegev	stems, flowers
	DM	stems, flowers
ASCLEPIADACEAE		
<i>Calotropis procera</i>	DM	whole plant
<i>Pentatropis nivalis</i>	DM	whole plant
BORAGINACEAE		
<i>Anchusa strigosa</i>	DM	leaves
	DM	prickles
	Kochla	stems, leaves, roots
	DM	stems, leaves, roots

Table 1. Cont'd

<i>Capparis spinosa</i>	Shimshonim hill Ein Mor	stems, leaves, flowers stems, leaves, flowers
CARYOPHYLLACEAE		
<i>Gymnocarpus decander</i>	DM	stems, leaves, flowers, fruit
<i>Gypsophila arabica</i>	DM	roots
	DM	stems, flowers
	Avdat	stems, flowers
	DM	bark
<i>Herniaria hemistemon</i>	DM	DM
<i>Paronychia arabica</i>	DM	whole plant
<i>Pteranthus dichotomus</i>	Sede Boqer Zin	stems, leaves stems, leaves, flowers
<i>Sclerocephalus arabicus</i>	DM	whole plant
<i>Spergula fallax</i>	DM	Pods
<i>Spergularia diandra</i>	DM	whole plant
CHENOPODIACEAE		
<i>Anabasis articulata</i>	DM	stems
<i>Atriplex halimus</i>	Kochla Sede Boqer Avdat	stems, leaves stems, leaves stems, leaves, flowers
<i>Atriplex holocarp</i>	DM	whole plant
<i>Atriplex leucocla</i>	DM	whole plant
<i>Bassia muricata</i>	DM	whole plant
<i>Chenopodium murale</i>	DM	whole plant
<i>Hammada scoparia</i>	Zin	stems
<i>Salsola baryosma</i>	DM	whole plant
<i>Salsola cyclophylla</i>	DM	whole plant
<i>Salsola damascena</i>	DM	whole plant
<i>Salsola gaetula</i>	DM	whole plant
<i>Suaeda aegyptiaca</i>	DM	whole plant
CISTACEAE		
<i>Helianthemum ventosum</i>	DM	stems, leaves
COMPOSITAE		
<i>Achillea fragrantissima</i>	DM	stems, leaves, flowers
<i>Achillea santolina</i>	DM	stems, leaves, flowers
<i>Artemisia judaica</i>	DM	whole plant
<i>Artemisia monosperma</i>	DM	whole plant
<i>Artemisia sieberi</i>	Kochla Zin	stems, leaves stems, leaves
<i>Asteriscus graveolens</i>	DM	whole plant
<i>Centaurea eryngioides</i>	Kochla	stems, leaves, flowers, roots
<i>Echinops polyceras</i>	DM	stems, leaves, fruit
<i>Gundelia tournefortii</i>	Kochla	stems, leaves, flowers, roots

Table 1. Cont'd

<i>Launaea nudicaulis</i>	DM	whole plant
<i>Phagnalon rupestre</i>	DM	DM
<i>Pulicaria crispa</i>	Ein Avdat	stems, leaves, flowers
	DM	stems, leaves, flowers
<i>Pulicaria incisa</i>	DM	whole plant
<i>Reichardia tingitana</i>	DM	whole plant
<i>Senecio glaucus</i>	DM	whole plant
CRUCIFERAE		
<i>Brassica tournefortii</i>	DM	whole plant
<i>Diplotaxis acris</i>	DM	whole plant
<i>Enarthrocarpus strangulatus</i>	DM	whole plant
<i>Moricandia nitens</i>	DM	stems, leaves, flowers
<i>Schimpera arabica</i>	DM	whole plant
<i>Sisymbrium irio</i>	DM	whole plant
EPHEDRACEAE		
<i>Ephedra aphylla</i>	Zin	stems
	Andartat Hanegev	stems, flowers
	Avdat	stems, flowers
	Zin	stems, flowers
GRAMINEAE		
<i>Phragmites australis</i>	DM	whole plant
<i>Schismus arabicus</i>	DM	whole plant
JUNCACEAE		
<i>Juncus arabicus</i>	DM	stems
LABIATAE		
<i>Ballota undulata</i>	Zin	stems, leaves, flowers
	Kochla	stems, leaves, flowers
<i>Salvia spinosa</i>	DM	stems, leaves, flowers, roots
	DM	stems, leaves
<i>Stachys aegyptiaca</i>	DM	stems & leaves & seeds ^b
LILIACEAE		
<i>Asparagus aphyllus</i>	DM	stems & fruit & seeds ^b
<i>Asphodelus tenuifolius</i>	DM	roots
<i>Urginea maritima</i>	DM	bulbs
LORANTHACEAE		
<i>Loranthus acacia</i>	Seif	leaves, branches
	Kochla	leaves, branches
MALVACEAE		
<i>Alcea acaulis</i>	DM	stems, leaves, flowers, fruit
<i>Lavatera cretica</i>	DM	whole plant

Table 1. Cont'd

<i>Malva nicaeensis</i>	DM	whole plant
<i>Acacia raddiana</i>	Kochla	Pods
	Kochla	leaves
	Seif	leaves
MIMOSACEAE		
<i>Teucrium capitatum</i>	Kochla	stems, leaves, flowers
	DM	stems
MOLLUGINACEAE		
<i>Glinus lotoides</i>	DM	whole plant
NEURADACEAE		
<i>Neurada procumbens</i>	DM	whole plant
OROBANCHACEAE		
<i>Cistanche tubulosa</i>	DM	flowers
PAPAVERACEAE		
<i>Glaucium arabicum</i>	DM	stems, leaves, flowers
PAPILIONACEAE		
<i>Alhagi graecorum</i>	DM	whole plant
<i>Medicago laciniata</i>	DM	whole plant
<i>Retama raetam</i>	Zin	stems
	Andartat Hanegev	stems, flowers
<i>Colutea istria</i>	Sede Boqer	stems, leaves, flowers
	DM	stems, leaves, flowers
POLYGONACEAE		
<i>Polygonum palaestinum</i>	Avdat	stems, flowers
	Andartat Hanegev	stems, leaves, flowers, roots
	Andartat Hanegev	stems, flowers
	Zin	stems, leaves, flowers
<i>Rumex cyprius</i>	DM	whole plant
<i>Rumex vesicarius</i>	DM	whole plant
POPILIONACEAE		
<i>Astragalus asterias</i>	DM	whole plant
RESEDACEAE		
<i>Caylusea hexagyna</i>	DM	stems
<i>Ochradenus baccatus</i>	Seif	branches, flowers
	DM	stems, flowers
	DM	stems, leaves, flowers
<i>Oligomeris linoifolia</i>	DM	whole plant
<i>Reseada stenostachya</i>	DM	whole plant

Table 1. Cont'd

SALANACEAE		
<i>Withania somnifera</i>	DM	whole plant
SALICACEAE		
<i>Populus euphratica</i>	DM	stems, leaves
SCROPHULARIACEAE		
<i>Kickxia aegyptiaca</i>	DM	stems, leaves, fruit
<i>Verbascum fruticosum</i>	Avdat	stems, leaves, flowers
	Kochla	stems, leaves, flowers
SOLANACEAE		
<i>Datura innoxia</i>	DM	whole plant
<i>Hyoscyamus reticulatus</i>	DM	stems, leaves, fruit
<i>Lycium shawii</i>	Andartat Hanegev	stems, leaves
	DM	stems, leaves
<i>Nicotiana glauca</i>	DM	stems, leaves, fruit
<i>Solanum elaeagnifolium</i>	DM	stems, leaves, fruit
<i>Solanum nigrum</i>	DM	whole plant
TAMARICACEAE		
<i>Reaumuria hirtela</i>	DM	stems, leaves, flowers
<i>Tamarix aphylla</i>	DM	whole plant
THYMELAEACEAE		
<i>Thymelaea hirsuta</i>	Kochla	stems, leaves
	Zin	stems, leaves
	Zin	stems, leaves, flowers
UMBELLIFERAE		
<i>Coriandrum sativum</i>	DM	seeds
<i>Ferula sinaica</i>	DM	leaves
	DM	roots
	DM	shoots
ZYGOPHYLLACEAE		
<i>Fagonia arabica</i>	DM	whole plant
<i>Fagonia glutinosa</i>	DM	whole plant
<i>Fagonia mollis</i>	DM	whole plant
<i>Peganum harmala</i>	DM	roots
	DM	seeds
	Kochla	stems, leaves, flowers
<i>Trigonella stellata</i>	DM	whole plant
<i>Zygophyllum album</i>	DM	whole plant
<i>Zygophyllum dumosum</i>	Andartat Hanegev	stems, leaves, flowers

DM = Data missing.

^a An extract was tested for each of the plant parts listed.

^b Extract was made for pooled plant parts.

Microplate Kit (Ilex Medical Systems, Hayward, California). *P. damsela*e was identified using the API-20E kit (bioMerieux sa, Marcy l'Etoile, France) and the latex bead agglutination kit (Bionor, Skien, Norway). Bacteria were stored at -80°C in 15% glycerol until used. *P. damsela*e was grown on BHI-A amended with 2% NaCl; the other bacteria were grown on BHI-A.

Plant material and extracts. Material was collected from 104 plant species that grow in the Negev. The material was oven dried at 40°C for five days, mechanically powdered, and stored at room temperature until tested. Plant samples included all plant parts or those available in sufficient quantity (approximately 2 g dry matter) at the time of collection. Dried plant powder was extracted with distilled de-ionized water at a ratio of 1:10 w/v by shaking for 4 h at 40°C. The extracts were centrifuged at 13,000 rpm at room temperature (Labofuge 400R, Heraeus, Germany) and the supernatant was stored at -20°C until analyzed.

Disk diffusion assay. Disk diffusion assay was conducted according to Alderman and Smith (2001). The surface of an agar plate was inoculated with a bacterial suspension, *A. hydrophila* and *V. alginolyticus* on Muller Hinton agar and *P. damsela*e and *S. iniae* on BHI-A. Sterile 6-mm paper filter disks were soaked with 22 µl of the aqueous plant extract (in duplicate) or a water control, placed on the inoculated agar plate, and incubated at 25°C for 24 h. The diameters of the bacterial growth inhibition were measured to an accuracy of 0.5 mm. Results are the average of the two measurements.

Results

Extracts of 17 plants had an inhibitory effect against at least one of the bacteria tested (Table 2) while there was no inhibition in the control. *Photobacterium damsela*e ssp. *piscicida* was inhibited by the largest number of plant extracts. High antibacterial activity (14-19.5 mm) against this species was obtained by *Achillea fragrantissima*, *Anchusa strigosa*, *Hammada scoparia*, *Loranthus acaciae*, and *Pulicaria crispa*. High inhibitory effect against *A. hydrophila* and *V. alginolyticus* was

Table 2. Antibacterial effect of plant extracts determined by the disk diffusion assay.

Family and species	Collection site	Plant parts	Growth inhibition (mm)			
			<i>A. hydrophila</i>	<i>P. damsela</i> e	<i>S. iniae</i>	<i>V. alginolyticus</i>
BORAGINACEAE						
<i>Anchusa strigosa</i>	Kochia	stems, leaves, roots	0	14	0	0
CHENOPODIACEAE						
<i>Hammada scoparia</i>	Zin	stems	13.75	14	0	12
COMPOSITAE						
<i>Achillea fragrantissima</i>	Kochia	stems, leaves, flowers	0	19.5	0	0
<i>Achillea santolina</i>	Kochia	stems, leaves, flowers	0	9	0	0
<i>Artemisia monosperma</i>	Wadi Nekarot	whole plant	0	7	0	0

Table 2. Con'd

<i>Asteriscus graveolens</i>	Hazeva	whole plant	0	8.75	0	0
<i>Gundelia tournefortii</i>	Kochia	stems, leaves, flowers, roots	0	7	0	0
<i>Pulicaria crispa</i>	Ein Avdat	stems, leaves, flowers	0	14	0	0
<i>Phagnalon rupestre</i>	Kochia	stems, leaves, flowers	0	7.5	0	0
LORANTHACEAE						
<i>Loranthus acaciae</i>	Seif	leaves, branches	7	15	0	7
MALVACEAE						
<i>Acacia raddiana</i>	Kochia	Pods	0	10.5	0	0
<i>Acacia raddiana</i>	Seif	leaves	0	10.5	0	0
MIMOSACEAE						
<i>Teucrium capitatum</i>	Kochia	leaves	0	8	0	0
OROBANCHACEAE						
<i>Cistanche tubulosa</i>	Zin	flowers	0	7.75	0	0
RESEDACEAE						
<i>Ochradenus baccatus</i>	Seif	branches, flowers	0	11.5	10.5	0
<i>Reseada stenostachya</i>	Wadi Ashosh	whole plant	0	11	10.5	0
THYMELAEACEAE						
<i>Thymelaea hirsuta</i>	Zin	stems, leaves	0	8	0	0
ZYGOPHYLLACEAE						
<i>Peganum harmala</i> ^b	Zin	seeds	18	0	0	20.5

^a An extract was tested for each of the plant parts listed.

^b Harmaline inhibition was determined in bacterial isolates that were sensitive to *P. harmala*.

obtained by *P. harmala* (18 and 20.5 mm, respectively).

A moderate inhibitory effect (<14 mm, >8 mm) of *P. damselae* was obtained by extracts of *Achillea santolina*, *Asteriscus graveolens*, *Acacia raddiana*, *Ochradenus baccatus*, and *Reseda stenostachya*. There was no difference in the inhibitory effect of pods and leaves of *A. raddiana* (10.5 mm). *Artemisia monosperma*, *Gundelia tournefortii*, *Phagnalon rupestre*, *Teucrium capitatum*, *Cistanche tubulosa*, and *Thymelaea hirsute* had low antibacterial effect against this bacterium (≤ 8 mm). *Hammada scoparia* had a moderate inhibitory effect (<14 mm, >8 mm) on *A. hydrophila* and *V. alginolyticus*. *Loranthus acaciae* had a low inhibitory effect (≤ 8 mm) on these bacteria. *Ochradenus baccatus* and *Reseda stenostachya* had a moderate inhibitory effect (10.5 mm) against *S. iniae*.

Some plants differed in effect according to the site of collection or plant part. The seeds of *P. harmala* were effective while the roots, stems, leaves, and flowers were not. Samples of *T. hirsuta* collected at Zin were effective while samples from Kochla were not. Samples of *A. strigosa*, *P. crispa*, *L. acacia*, *A. raddiana*, *T. capitatum*, and *O. baccatus* collected from different sites and containing different plant parts also differed in effect.

Discussion

Extracts of 104 desert plants were screened for antibacterial activity against pathogenic bacteria in fish. The disk diffusion assay was a rapid and simple technique for screening a large number of samples. Extracts from 17 of the tested species inhibited growth of at least one of the tested bacteria. None of the plant extracts inhibited all the bacteria.

Peganum harmala seed extract had the highest inhibitory activity of any of the plant extracts while the root, stem, leaf, and flower extracts had no inhibitory effect. Seeds of *P. harmala* are known to contain biologically active compounds, i.e., β -carbolines and alkaloids such as harmaline, harmine, and harmalol, which were reported to have antibacterial properties (Kashimov et al., 1971; Al-Shamma et al., 1981; Siddiqui et al., 1990). Commercial harma-

line inhibited the growth of *A. hydrophila* and *V. alginolyticus* (13 and 16 mm, respectively) when applied at approximately 100 $\mu\text{g}/\text{disk}$ (data not shown), which in part might be responsible for the inhibitory effect of *P. harmala*.

High antibacterial activity was also obtained from extracts of *A. fragrantissima* and *A. strigosa*, which inhibited the growth of *P. damselae* by 19.5 mm and 14 mm, respectively. The inhibitory activity in *A. fragrantissima* might be related to the presence of monoterpenes such as camphor, 1,8-cineole, and borneol that were identified in the genus *Achillea* (Chalchat et al., 2000; Simic et al., 2000) and are known as antimicrobial agents (Lis-Balchin and Deans, 1997). Antibacterial and antifungal activity of *A. strigosa* extract was reported by Nadir et al. (1986); this plant was used to treat stomach ulcers (Disi et al., 1998).

The extracts of *P. crispa*, *L. acaciae*, and *H. scoparia* inhibited *P. damselae* growth by 14-15 mm. Reference to the biological activity in these species is found in ethnobotanical literature (e.g., Al Yahya et al., 1988; Sathiyamoorthy et al., 1999). *P. crispa* contains cancer-preventive and cytotoxic compounds (Al-Yahya et al., 1988).

Different samples of some of the tested plant species varied in their antibacterial effect. Potential sources of the variation are differences in plant part, site, or season of collection of the samples. It can be hypothesized that the antibacterial activity detected in *P. harmala* was due to the β -carbolines in the seed (Al-Shamma et al., 1981) while the variability in the activity of *T. hirsuta* was due to seasonal variation. Accumulation of secondary metabolites varies at different times of the year, and is related to availability of water, temperature, and day length (Croteau et al., 2000).

Strict regulations limit the use of antibiotics and other chemicals in aquaculture (Alderman and Hastings, 1998). The use of desert plants as an alternative treatment for controlling bacterial diseases in fish is potentially important in producing organically grown fish. More research is needed to identify the active compounds, determine toxicity to fish and humans, and develop formulations.

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