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SCREENING OF DESERT PLANTS FOR USE AGAINST BACTERIAL PATHOGENS IN FISH

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Abstract

The antibacterial activity of aqueous extracts of 104 desert plant species was studied on the following fish bacterial pathogens: *Aeromonas hydrophila*, *Photobacterium damselae* subspecies *piscicida*, *Streptococcus iniae*, and *Vibrio alginolyticus*. Seventeen plant species had antibacterial activity, as identified by disk diffusion assay. The pathogen *P. damselae* 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 damselae* 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 part*[a]
AIZOACEAE		
Aizoon canariense	DM	whole plant
Mesembryanthemum nodiflorum	Ein Mor	stems, leaves, flowers
	Zin	stems, leaves
AMARANTHACEAE		
Aerva javanica	DM	whole plant
APIACEAE		
Pituranthos tortuosus	DM	bulbs
	Andartat Hanegev	stems, flowers
	DM	stems, flowers
ASCLEPIADACEAE		
Calotropis procera	DM	whole plant
Pentatropis nivalis	DM	whole plant
BORAGINACEAE		
Anchusa strigosa	DM	leaves
	DM	prickles
	Kochla	stems, leaves, roots
	DM	stems, leaves, roots

Table 1. Cont'd

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

Table 1. Cont'd

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

Table 1. Cont'd

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

Table 1. Cont'd

SALANACEAE		
Withania somnifera	DM	whole plant
SALICACEAE		
Populus euphratica	DM	stems, leaves
SCROPHULARIACEAE		
Kickxia aegyptiaca	DM	stems, leaves, fruit
Verbascum fruticulosum	Avdat	stems, leaves, flowers
	Kochla	stems, leaves, flowers
SOLANACEAE		
Datura innoxia	DM	whole plant
Hyoscyamus reticulatus	DM	stems, leaves, fruit
Lycium shawii	Andartat Hanegev	stems, leaves
	DM	stems, leaves
Nicotiana glauca	DM	stems, leaves, fruit
Solanum elaeagnifolium	DM	stems, leaves, fruit
Solanum nigrum	DM	whole plant
TAMARICACEAE		
Reaumuria hirtela	DM	stems, leaves, flowers
Tamarix aphylla	DM	whole plant
THYMELAEACEAE		
Thymelaea hirsuta	Kochla	stems, leaves
	Zin	stems, leaves
	Zin	stems, leaves, flowers
UMBELLIFERAE		
Coriandrum sativum	DM	seeds
Ferula sinaica	DM	leaves
	DM	roots
	DM	shoots
ZYGOPHYLLACEAE		
Fagonia arabica	DM	whole plant
Fagonia glutinosa	DM	whole plant
Fagonia mollis	DM	whole plant
Peganum harmala	DM	roots
	DM	seeds
	Kochla	stems, leaves, flowers
Trigonella stellata	DM	whole plant
Zygophyllum album	DM	whole plant
Zygophyllum dumosum	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. damselae* 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. damselae* 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 deionized 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. damselae* 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 damselae* 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 part*[a]	*Growth inhibition (mm)*			
			A. hydrophila	*P. damselae*	*S. iniae*	*V. alginolyticus*
BORAGINACEAE						
Anchusa strigosa	Kochla	stems, leaves, roots	0	14	0	0
CHENOPODIACEAE						
Hammada scoparia	Zin	stems	13.75	14	0	12
COMPOSITAE						
Achillea fragrantissima	Kochla	stems, leaves, flowers	0	19.5	0	0
Achillea santolina	Kochla	stems, leaves, flowers	0	9	0	0
Artemisia monosperma	Wadi Nekarot	whole plant	0	7	0	0

Table 2. Con'd

Asteriscus graveolens	Hazeva	whole plant	0	8.75	0	0
Gundelia tournefortii	Kochla	stems, leaves, flowers, roots	0	7	0	0
Pulicaria crispa	Ein Avdat	stems, leaves, flowers	0	14	0	0
Phagnalon rupestre	Kochla	stems, leaves, flowers	0	7.5	0	0
LORANTHACEAE						
Loranthus acaciae	Seif	leaves, branches	7	15	0	7
MALVACEAE						
Acacia raddiana	Kochla	pods	0	10.5	0	0
Acacia raddiana	Seif	leaves	0	10.5	0	0
MIMOSACEAE						
Teucrium capitatum	Kochla	leaves	0	8	0	0
OROBANCHACEAE						
Cistanche tubulosa	Zin	flowers	0	7.75	0	0
RESEDACEAE						
Ochradenus baccatus	Seif	branches, flowers	0	11.5	10.5	0
Reseada stenostachya	Wadi Ashosh	whole plant	0	11	10.5	0
THYMELAEACEAE						
Thymelaea hirsuta	Zin	stems, leaves	0	8	0	0
ZYGOPHYLLACEAE						
Peganum harmala [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 harmaline inhibited the growth of *A. hydrophila* and *V. alginolyticus* (13 and 16 mm, respectively) when applied at approximately 100 µg/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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