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Antimicrobial and Cytotoxic Activity of Some Medicinal Plants from Baja California Sur (Mexico)

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Abstract

Scientific evaluation of 25 ethanol extract of plants used in the traditional medicine of Baja California Sur (Mexico) were tested for microbial and HCT-116 cell growth inhibition. Ten extracts showed activity against the HCT-116 cell line, notably *Asclepias subulata* (Asclepiadaceae), *Aristolochia brevipes* (Aristolochiaceae) and *Bursera odorata* (Burseraceae). *Haplopappus sonorensis* (Asteraceae) *Asclepias subulata* and *Bursera odorata* inhibited *Mycobacterium tuberculosis* by 40, 45 and 67%, respectively, at a concentration of 100 µg/mL. Antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis, Candida albicans* and *Escherichia coli* was determined, and *Aristolochia monticola*, *A. brevipes, Hymenoclea monogyra* and *Hymenoclea* sp. were found to be the most active. *Xanthium strumarium* showed low activity against *C. albicans.*

Keywords: Antimicrobial, antimycobacterial, Baja California Sur, México, cytotoxicity, medicinal plants.

Latin binomials with authority and family
Aff *Capsella* sp. BRACICACEAE
Ambrosia psilostachya DC. ASTERACEAE
Anaphalis margaritacea (L.) A. Gray ASTERACEAE
Aristolochia brevipes Brandegee ARISTOLOCHIACEAE
Aristolochia monticola Brandegee ARISTOLOCHIACEAE
Asclepias subulata Decne. ASCLEPIADACEAE
Avicennia nitida Jacp. AVICENNIACEAE
Baccharis glutinosa Pers. ASTERACEAE
Baccharis sp. ASTERACEAE
Bidens pilosa var. *radiata* Schultz-Bip. ASTERACEAE
Bursera odorata Brandegee BURSERACEAE
Crescentia alata HBK BIGNONIACEAE
Descurainia pinnata subsp. *menziesii* (DC.) Detling BRACICACEAE
Elytraria imbricata (Vahl) Pers ACANTHACEAE
Foenicullum vulgare Mill APIACEA
Gnaphalium purpureum L. ASTERACEAE
Haplopappus sonorensis (A. Gray) S. F. Blake ASTERACEAE
Haplopappus spinulosus (Pursh) DC. Subsp. *scrabellus* (Greene) Hall ASTERACEAE
Heliotropium curassavicum L. var. *oculatum* (Heller) I. M. Jhth. BORAGINACEAE
Hymenoclea monogyra Torr & Gray ASTERACEAE
Hymenoclea sp. ASTERACEAE
Jacobina spicigera (Schlecht) Baily ACANTHACEAE
Pectis hankeana D.C. Schu. Bip. ASTERACEAE
Spondias sp. ANACARDIACEAE
Xanthium strumarium L. ASTERACEAE

Introduction

Epidemiological statistics show that cancer and infectious diseases are important causes of morbidity and mortality throughout the world (WHO, 1999). Many of these health problems could be avoided by improving sanitation conditions or better management of illness. The emergence of multidrug-resistant pathogens have been well-documented (Davies, 1994; Nikaido, 1994; Spratt, 1994). Consequently, patients infected with resistant strains are extremely difficult to cure, and treatment is much more toxic and expensive. Outbreaks of multidrug-resistant tuberculosis in developed and developing countries have focused global attention on *Mycobacterium tuberculosis* resistant to antimycobacterial agents (WHO, 1997). Drug resistance is also a problem with cancer cells (Larsen, 1996). For this reason, new antiinfective and anticancer agents need to be developed. In search-

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ing for new antibiotics, biological screening and bioassay/guided separation of medicinal plants extracts are well-accepted methods for determining the active constituents responsible for the effectiveness of herbal remedies. The chemodiversity offered by plants is a very promising source for new compounds in the areas of cancer and infectious diseases (Yue-Zhong, 1998).

In our effort to contribute in this field, we have investigated the antimycobacterial and cytotoxic activity of 25 ethanol extracts from plants used to treat infectious diseases in the traditional medicine of Baja California Sur (México). These plants were stored and kept dry for several years (Encarnación & Contreras, 1992; Encarnación & Agúndez, 1995). Plants were selected as a result of previous screening where they showed interesting antimicrobial activity (Encarnación & Keer, 1991; Encarnación et al., 1998). In addition to this new antimycobacterial and cytotoxic screening, the antimicrobial activity previously reported with this same group of plant extracts was assayed again. The low cost and simple technique of the agar diffusion bioassay is advantageous for determining the antibacterial activities of crude extracts. This classic method requires that the test substances be soluble in water, or can diffuse into agar. Accordingly, antibacterial activity was determined using the agar-diffusion method with filter paper discs (Encarnación & Keer, 1991; Rios et al., 1988). Antimycobacterial bioassays were performed using the BACTEC 460 system (Cantrell et al., 1998; Collins & Franzblau, 1997), and cytotoxicity was tested with HCT-116 cells.

Materials and methods

Plant material

Plants used for extraction in this study were obtained from the dry medicinal plants collection of the Pharmacognostical Research Program of Universidad Autónoma de Baja California Sur (Table 1). Plants were collected between October of 1986 and March 1993 from different localities of Baja California Sur (Mexico). Each specimen has been kept in a glass flask labeled, numbered, and annotated with the date of collection, the locality, and the medicinal use. The plant material was previously identified by Jorge Agúndez from the Agronomy Department of UABCS. A set of herbarium specimens was retained at the Pharmacognosy Laboratory of Universidad Autónoma de Baja California Sur as reference. Voucher specimens were deposited from 1986 to 1993 at the herbarium of the Biology Institute of the Universidad Nacional Autónoma de Mexico, Mexico City.

Preparation of ethanol extracts

To prepare ethanol extracts for biological screening, 20 g of dried and ground plant material was macerated for 24 h with 100 mL × 2 of distilled ethanol, then filtered and concentrated to dryness *in vacuo* at 40 °C.

Microorganisms and inoculum preparation

Bacillus subtilis, Streptococcus faecalis (Gram-positive), *Escherichia coli* (ATCC 25922) (Gram-negative) and *Candida albicans* (yeast) were supplied by SCRIPPS Institution of Oceanography of the University of California, San Diego, California, U.S.A. *Staphylococcus aureus* was supplied by QBP Ana Ma. Ramírez from Laboratorio de Análisis Especiales, La Paz, B.C.S. Bacteria were cultured in 5 mL of nutrient broth (Gram-positive and Gram-negative microorganisms) or 5 mL of Saboraud broth (yeast) for 24 h at 37 °C and then adjusted to match the turbidity of a McFarland #5 standard. The growth and purity of each suspension was verified by Gram stain.

Mycobacterium tuberculosis $H_{37}Rv$ (ATCC 27294, American Type Culture Collection, Rockville, MD) was cultured at 37°C on a rotary shaker in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 0.2% v/v glycerol and 0.05% v/v Tween 80 until the culture turbidity achieved an optical density of 0.45–0.55 at 550 nm. Bacteria were centrifuged and the pellet washed twice and resuspended in one-fifth the original volume in Dulbecco's phosphat-buffered saline (PBS, Irvine Scientific, Santa Ana, CA). Large bacterial clumps were removed by passage through an 8 µm filter (Nalgene, Rochester, NY) and aliquots were frozen at −80 °C.

M. avium (ATCC 25291) was cultured in BACTEC 12B broth until a daily GI of 999 was reached. Cultures were then diluted 1 : 25 in BACTEC 12B broth and frozen al −80 °C until needed.

Antibacterial testing by the agar diffusion method

The ethanol extract of each plant (20 mg) was dissolved in 1.0 mL of the proper solvent and the solutions were used to impregnate a filter paper disk (6 mm) with 100 µL and dried at room temperature to have a final concentration of 2.0 mg/disk.

The test was done by placing the disk impregnated with the ethanol crude extracts on the agar surface previously inoculated with a sterile hyssop containing a suspension of each type of microorganism. The bacteria were cultured in Muller-Hinton Agar; Dextrosa Saboraud Agar was used to cultivate *C. albicans*. The growth and purity of each culture were verified by use of a Gram stain procedure.

Chloramphenicol and nalidixic acid were used as positive controls and the solvent was a negative control.

Plates were incubated at 37 °C for 24 h and the inhibition zones around the disks were measured and recorded at the end of the incubation period.

Antimycobacterial bioassay

Antimycobacterial testing was performed using the BACTEC 460 system (Cantrell et al., 1998). Stock solutions and subsequent dilutions were prepared at 80 × the final

Table 1. Antimicrobial and cytotoxic activity of ethanol extracts of medicinal plants from Baja California Sur (Mexico).

FAMILY	Agar diffusion test Inhibition zone at 2.0 mg/disk[a]					Antimycobacterial test % Inhibition at 100 µg/mL		HCT-116 cell line IC_{50} (µg/mL)
(Voucher Specimen) Latin Binomials and Authority	A	B	C	D	E	F	G	H
ACANTHACEAE								
(E-40) *Elytraria imbricata* (Vahl) Pers.	–	–	–	–	–	24	–	–
(E-51) *Jacobina spicigera* (Schlecht) Baily	–	–	–	–	–	–	–	84.3
ANACARDIACEAE								
(E-244) *Spondias* sp.	++	–	++	–	–	–	–	–
APIACEA								
(E-221) *Foenicullum vulgare* Mill	–	–	–	–	–	–	–	–
ARISTOLOCHIACEAE								
(E-36) *Aristolochia brevipes* Brandegee	+++	+	+	–	–	17	–	1.0
(E-269) *Aristolochia monticola* Brandegee	++++	+	+++	–	–	–	–	19.7
ASCLEPIADACEAE								
(E-1) *Asclepias subulata* Decne.	–	–	–	–	–	45	–	0.4
ASTERACEAE								
(E-21) *Ambrosia psilostachya* DC.	+	++	–	–	–	33	–	81
(E-17) *Anaphalis margaritacea* (L.) A. Gray	++	–	–	–	–	–	–	–
(E-12) *Baccharis glutinosa* Pers.	+	–	–	–	–	–	–	–
(E-34) *Baccharis* sp.	+	++	–	–	–	33	8	–
(E-28) *Bidens pilosa* var. *radiata* Schultz-Bip.	–	–	–	–	–	16	–	71
(E-279) *Gnaphalium purpureum* L.	+	–	+	–	–	3	–	–
(E-46) *Haplopappus sonorensis* (A. Gray) S. F. Blake	+	–	–	–	–	40	–	35.9
(E-240) *Haplopappus spinulosus* (Pursh) DC. subsp. *scrabellus* (Greene) Hall	–	–	–	–	–	–	–	–
(E-274) *Hymenoclea monogyra* Torr. & Gray	+	++++	–	–	+	13	–	–
(E-272) *Hymenoclea* sp.	+	+++	–	–	–	26	–	–
(E-176) *Pectis hankeana* D.C. Schu. Bip.	+	–	–	–	–	–	–	–
(E-219) *Xanthium strumarium* L.	+	–	+	–	+	18	–	84.6
AVICENNIACEAE								
(E-192) *Avicennia nitida* Jacp.	–	–	–	–	–	–	–	–
BIGNONIACEAE								
(E-131) *Crescentia alata* HBK	–	–	–	–	–	23	–	–
BORAGINACEAE								
(E-23) *Heliotropium curassavicum* L. var. *oculatum* (Heller) I. M. Jhtn	–	–	–	–	–	–	–	–
BRACICACEAE								
(E-327) *Descurainia pinnata* subsp. *menziesii* (DC.) Detling	–	–	–	–	–	38	–	67
(E-246) Aff *Capsella* sp.	+	–	–	–	–	–	–	–
BURSERACEAE								
(E-58) *Bursera odorata* Brandegee	–	–	–	–	–	67	–	<0.076

A = *Bacillus subtilis*
B = *Staphylococcus aureus*
C = *Streptococcus faecalis*
D = *Escherichia coli*
E = *Candida albicans*
F = *Mycobacterium tuberculosis*
G = *Mycobacterium avium*
H = Human colorectal cancer cell line (HCT-116)
[a] Grading of results: –, no inhibition
+, inhibition zone <10 mm in diameter.
++, inhibition zone of 10 to 13 mm in diameter.
+++, inhibition zone of 13 to 15 mm in diameter.
++++, inhibition zone of 15 to17 mm in diameter.
+++++, inhibition zone > 17 mm in diameter.

desired concentration in dimethylsulfoxide (DMSO) and sterilized by passage through 0.22 µm PFTE filters (Millex-FG, Millipore, Bedford, MA). Fifty microliters of test samples were added to 4 mL of BACTEC 12B medium (Becton Dickinson). Controls received 50 µL DMSO, producing a final concentration of 1.25% v/v. Rifampin (Sigma Chemical Co., St. Louis. MO) and clarithromycin (Abbott Labs, North Chicago, IL) were included as positive drug controls for *M. tuberculosis* and *M. avium*. They were solubilized and diluted to achieve a range of concentrations for determination of minimum inhibitory concentrations. Cultures were inoculated with approximately 4×10^5 cfu in a volume of 100 µl. For determination of percent inhibition, cultures were incubated at 37 °C and the growth index (GI; one GI unit = 0.25 nCi CO_2) determined daily in a BACTEC 460 instrument until control cultures achieved a GI of 999. Additional control vials were included which received a further 1 : 100-diluted inoculum of *M. tuberculosis* and *M. avium* for use in calculating the MIC of rifampin and clarithromycin by established procedures (Inderlied & Nash, 1996). Assays were usually completed in 5–8 days for *M. tuberculosis* and 6 days for *M. avium*. Percent inhibition was defined as 1 – (GI of test sample/GI of control) × 100. For determination of MIC values, the GI was determined daily until the GI of the 1 : 100 controls was at least 30. All vials were read the following day and the daily change in GI (ΔGI) was recorded for each drug dilution. The MIC was defined as the lowest concentration for which the ΔGI was less than the ΔGI of the 1 : 100 control (Inderlied & Nash, 1996).

Cytotoxicity test

Cytotoxicity was tested on HCT-116 (human colorectal cancer) cells. The cells were maintained in McCoys 5A medium containing 20% of fetal bovine serum and 10% DMSO. The cells were cultured in microtiter plates at 37 °C in a humidified atmosphere of 5% CO_2 in air. Samples dissolved in DMSO were added. The cells were incubated under the same conditions for 72 h. At the end of the incubation period, cells were fixed and stained with 0.0075% crystal violet. IC_{50} was defined as the point where cells begin to die, as judged by color changes from solid blue to clear.

Results and Discussion

The results of this screening are summarized in Table 1. *B. subtilis, S. aureus* and *S. faecalis* were the most susceptible microorganisms. Fourteen ethanol extracts inhibited *B. subtilis*, from which six were active against *S. aureus* as well as were active *Ambrosia psilostachya*, *Baccharis* sp. *Hymenoclea monogyra* and *Hymenoclea* sp., and two were active against *S. faecalis*; *Spondias* sp. and *Aristolochia monticola*. None of the tested extracts inhibited *E. coli*. Extracts from *Xantium strumarium* and *Hymenoclea monogyra* displayed activity against *C. albicans*. *Aristolochia brevipes* and *Aristolochia monticola* were active against the three Gram-positive bacteria. These last two plants showed significant cytotoxicity against the HCT-116 cell line at 1.0 and 19.7 µg/mL, respectively. Species of the *Aristolochia* genus are known to contain highly cytotoxic aristolochic acids and derivatives (Hashimoto et al., 1999), and this might explain the biological activity shown by this screening.

Sixteen extracts displayed some effect on *M. tuberculosis*, but only those where inhibition was higher than 40% at 100 µg/mL were considered active. Thus, *Haplopappus sonorensis*, *Asclepias subulata*, and *Bursera odorata* were active against *M. tuberculosis* showing inhibitions of 40, 45, and 67% at a concentration of 100 µg/mL, respectively, in the radiometric assay, and they were cytotoxic against HCT-116 cells.

The HCT-116 cell line was inhibited by 10 ethanol extracts (37%). Extracts from *A. subulata* and *A. brevipes* were highly cytotoxic with IC_{50} values of 0.4 and 1.0 µg/mL, respectively. The highest cytotoxicity was shown by the *B. odorata* extract, with a $IC_{50} < 0.076$ µg/mL. This plant is reported in the Traditional Medicine of B.C.S. as toxic and the exudate gum is used to kill dogs. *A. subulata* and *A. brevipes* belong to a family rich in cardiac glycosides which are toxic. Eighty percent of the cytotoxic extracts displayed some inhibition of *M. tuberculosis.* This high correlation between cytotoxic effect and inhibition of *M. tuberculosis*, could suggest some mechanistic relationship between both inhibitory effects.

From this group of plants, the fresh material of 18 were previously tested for antimicrobial activity (Encarnación & Keer, 1991; Encarnación et al., 1998). We have found differences in reported activity. It may be that in the previous screenings the extracts were tested at a higher concentration (2.8 mg/disk), and the fresh material could be more active due to the presence of active volatile compounds, that diminish in storage for several years. This could explain the activity we observed with *Elytraria imbricata*, *Jacobinia spicigera*, *Faeniculum vulgare*, *Asclepias subulata*, *Bidens pilosa*, *Haploppapus spinulosus*, *Avicennia nitida*, and *Crescentia alata*, in which the ethanol extracts did not present any activity. However, we found some reproducible results against one or two of the bacteria tested in the case of *Spondias* sp., *Aristolochia brevipes*, *Ambrosia psilostachya*, *Anaphalis margaritacea*, *Baccharis glutinosa*, *Hymenoclea monogyra*, and *Xanthium strumarium.*

For example, the branches of *Haplopappus sonorensis* (A. Gray) S.F. Blake (Asteraceae), known locally as "Hierba del Pasmo", are used in Traditional Medicine of B.C.S. (México) against skin ulcer, cold, general infections, heart troubles, headache, toothache, cough "tetanus", wounds, and bad smelling feet. In preliminary studies carried out with a group of plants in which fresh material was used, *Haplopappus sonorensis* showed activity against *S. aureus*, *B. subtilis*, and *S. faecalis* (Encarnación & Keer, 1991). We observed the activity of the same material diminished with the dried material stored for several years. When we placed a lower

concentration on the disk (2 mg/disk), activity against *S. aureus* was observed. This is an interesting plant, and antimycobacterial and cytoxicity compounds isolated from this plant will be published later in a separate paper.

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References

Cantrell CL, Fischer NH, Orbatch L, McGuire MS, Franzblau SG (1998): Antimycobacterial crude plant extracts from South Central North America. *Phytomedicine 5*: 139–147.

Collins L, Franzblau S (1997): Microplate Alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrobial Agents Chemother 41*: 1004–1009.

Davies J (1994): Inactivation of antibiotics and the dissemination of resistance genes. *Science 264*: 375–382.

Encarnación R, Agúndez J (1995): Medicina tradicional de Baja California Sur. *Revista Mexicana Cienc Farm 26*: 25–44.

Encarnación R, Contreras G (1992): Medicina tradicional de Baja California Sur (México) IV. *Revista Médica del IMSS 30*: 297–308.

Encarnación R, Keer S (1991): Antimicrobial screening of medicinal plants from Baja California Sur. *J Ethnopharmacol 31*: 181–192.

Encarnación R, Virgen M, Ochoa N (1998): Antimicrobial activity of medicinal plants from Baja California Sur (México). *Pharm Biol 36*: 33–43.

Hashimoto K, Higuchi M, Sakakibara I, Kubo M, Komatsu Y, Maruno M, Okada M (1999): Quantitive analysis of aristolochia acids, toxic compounds contained in some medicinal plants. *J Ethnopharmacol 64*: 185–189.

Inderlied CB, Nash KA (1996): Antimycobacterial agents: *in vitro* susceptibility testing, spectra of activity, mechanisms of action and resistance, and assay for activity in biological fluids. *In*: V. Lorian (ed.) *Antibiotics in Laboratory Medicine*. Williams and Wilkens, Baltimore. pp. 127–175.

Larsen IK (1996): Anticancer agents. *In*: Krogsgaard P, Liljefors T, Madsen U eds., *A Textbook of Drug Design and Development.* The Netherlands, Harwood Academic Publisher, pp. 460–507.

Nikaido H (1994): Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science 264*: 382–388.

Rios JL, Rocío MC, Villar A (1998): Screening methods for natural products with antimicrobial activity: a review of the literature. *J Ethnopharmacol 23*: 127–129.

Yue-Zhong S (1998): Recent natural products based drug development: a pharmaceutical industry perspective. *J Nat Prod 61*: 1053–1071.

Spratt BG (1994): Resistance to antibiotics mediated by target alterations. *Science 264*: 388–393.

WHO (1999): World Health Report 1998. Office of World Health Reporting. Geneva, Switzerland

WHO/IUATLD Global project on anti-tuberculosis drug resistance surveillance 1994–1997 (1997): Anti-tuberculosis drug resistance in the world. Geneva, Switzerland. pp. 17–18.