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ANTIMICROBIAL SCREENING OF MEDICINAL PLANTS FROM BAJA CALIFORNIA SUR, MEXICO

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Summary

The ethanolic extracts of 72 plants belonging to 35 different families, and used in traditional medicine in Baja California Sur (México), were tested for antimicrobial activity in vitro using the filter paper disk assay method. Activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* (Gram-positive microorganisms), *Escherichia coli* (Gram-negative microorganisms) and *Candida albicans* (yeast) is discussed.

Introduction

Many kinds of diseases have been treated with herbal medications throughout the history of mankind. Herbal preparations still continue to be used extensively in many countries such as Mexico, where millions of people are still using them today to control and cure sickness. It is a necessity, from the scientific point of view, to establish a rational relationship between chemical composition and biological and therapeutic activities. That is the reason for our interest in evaluating the plant sources used in the traditional medicine of Baja California Sur, Mexico.

The search for biologically active compounds from natural sources has always been of great interest to scientist looking for new sources for drugs useful in infectious deseases. In recent years a number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Malcolm and Sofowora, 1969; Bhakuni et al., 1974; Taniguchi et al., 1978; Moskalenko, 1986) and marine organisms (Green, 1977; Berquist, 1978; Amade, 1982; Anderson et al., 1983).

In Baja California Sur very little research has been carried out in the field of natural products chemistry directed towards the isolation and structure elucidation of active compounds. This shortcoming, together with the fact that Baja California Sur has a rich tradition in the uses of medicinal plants

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(Encarnación and Agundez, 1986; Encarnación et al., 1987) motivated the initiation of this project, with the goal of isolating useful active compounds present in the natural sources of this area.

Considering that many of the medicinal plants reported in the traditional medicine of Baja California Sur are used against bacterial infections (Encarnacion and Agundez, 1986; Encarnación et al., 1987), antimicrobial screening of the ethanolic crude extracts of these natural sources has been performed. This paper presents the results of what may be considered to be the first systematic attempt to study the medicinal plants of this geographic area for antibacterial activity.

Material and Methods

Collection of plant material

Plants reported used in the traditional medicine of Baja California Sur were collected from different localities of the region (Encarnación and Agundez, 1986). Each specimen was labeled, numbered, annotated with the date of collection, the locality and the medicinal use. A set of herbarium specimens was retained at the Marine Biology Department of the Universidad Autonoma of Baja California Sur (Mexico) for identification. Duplicate specimens were deposited at the herbarium of the Biology Institute, Universidad Nacional Autonoma of Mexico, Mexico City.

Preparation of extracts and disks

In order to prepare initial extracts for biological testing, one part of dried material with about five of ethanol was macerated for 8 days. Since the purpose of our study was qualitative determination not quantitative, weight and volume of extraction were not recorded. The ethanolic extracts were evaporated at room temperature (no more than 40 °C) and 20 mg of the dry residue of each sample was again dissolved in 1 ml of ethanol. Filter paper disks (7 mm diameter) were impregnated with 140 µl of each solution and dried at room temperature.

Microorganisms

Five different laboratory bacterial strains were used, namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* (Gram-positive), *Escherichia coli* (Gram-negative) and *Candida albicans* (yeast). The microorganisms were supplied by Mark Roman of the SCRIPPS Institution of Oceanoraphy of the University of California, San Diego, California, U.S.A.

Growth medium

Screening was performed on plates of peptone agar (15–20% agar, pH 7.0 –7.4), sterilized for 15 min at 120 °C. Approximately 20 ml of this medium was added to each 100-mm sterile petri dishes and kept for 24 h to control sterility.

Antibacterial testing

All tests were done by placing the disks impregnated with the ethanolic crude extracts on the agar surface previously inoculated with a sterile hyssop containing a suspension of each type of microorganisms. The suspension was inoculated in 5 ml of nutrient agar liquid (Gram-positive and Gram-negative microorganisms) and 5 ml of Sabouraud liquid (yeast), and incubated for 24 h at 37 °C. The growth and purity of each suspension was verified by using a Gram stain. Standard disks of chloramphenicol (30 μg/disk), erythromycin (15 μg/disk) and nalidixic acid (30 μg/disk) were used as reference (positive) controls. Disks with evaporated ethanol used for the preparation of plant extracts served as a negative control. Plates were incubated at 37 °C for 24 h and zones of inhibition around the disk were measured at the end of the period.

Results

The antimicrobial activity of the crude ethanolic extracts of 72 medicinal plants used in the traditional medicine of Baja California Sur were tested. The results are presented in Table 1. Most of the extracts were active against Gram-positive microorganisms. Among these, 55 (76.3%) extracts were active against *Staphylococcus aureus*, 43 (59.7%) against *Bacillus subtilis* and 21 (29.1%) against *Streptococcus faecalis*. Three extracts (4%) were active against *Escherichia coli* (Gram-negative) and 13 (18%) against *Candida albicans* (yeast).

As can be seen from Table 1, the antimicrobial screening was performed in the majority of cases on the branch sample, followed by the whole plant and the root samples. The choice of sample to screen was dictated by the informants who indicated which plant part was the part used in their preparations. It was not always possible to collect adequate amounts of material during the interviews for screening and for herbarium specimens; consequently, the amounts used for extraction were variable. Since the purpose of the screening was qualitative, the results presented in Table 1 have satisfied the objective of the present investigation.

The amount of secondary metabolites in plant can vary with the season, the part of the plant and the type of the soil (Tyler et al., 1976; Anand and Nityanand, 1984). Based on this consideration, both native and introduced species were screened for their antimicrobial activity. During extraction, the ambient temperature was mantained between 30° and 40 °C to avoid changes in the amount and structure of the active compounds. The humidity of the room was not a factor due to the dryness of the working area.

From Table 1, it may be seen that:

(1) The most active extracts against *Staphylococcus aureus* are from *Bursera microphylla* (Burseraceae), *Wislizenia refracta* (Capparidaceae), *Baccharis glutinosa* (Compositae), *Pithecellobium dulce* (Mimosaceae), *Fraxinus uhlei* (Olacaceae), *Ludwigia octovalvis* (Onagraceae), *Pellaea*

TABLE 1

ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS FROM BAJA CALIFORNIA SUR, MEXICO

Family Botanical name (Voucher specimen)	Plant part tested	Antibacterial activity[a] A[b]	B	C	D	E
ACANTHACEAE						
Elitraria imbricata (Vahl) Pers. E 40)[c]	Whole plant	+	+	+	–	–
Jacobinia spicigera (Shlecht.) Bailey (E 51)	Branches	+ +	+	–	–	–
ANACARDIACEAE						
Rhus laurina Nutt. (E 35)	Branches	+ +	+ +	+ +	–	–
ARISTOLOCHIACEAE						
Aristolochia brevipes Benth. (E 36)	Root	+ +	+ + + +	–	–	–
ASCLEPIADACEAE						
Asclepias subulata Decne. (E 1)	Root	+	+	+ +	–	–
BURSERACEAE						
Bursera microphylla A. Gray (E 67)	Branches	+ + +	+	–	–	+
Bursera odorata Brandegee (E 58)	Branches	+ +	+ +	+ +	–	–
CAESALPINIACEAE						
Cassia confinis Greene (E 29)	Branches	+ +	+	–	–	–
Haematoxylon brasiletto Karst. (E 26)	Branches	+ +	–	+ +	–	–
Parkinsonia aculeata L. (E 56)	Branches	+ +	+ + +	+	–	–

CAPPARRIDACEAE						
Atamisquea emarginata Miers (E 61)	Branches	+ +	+ +	−	−	−
Wislizenia refracta Engelm var. *palmeri* (A. Gray) I. M. Johnston (E 31)	Branches	+ + +	−	−	+	−
CAPRIFOLIACEAE						
Sambucus mexicana Presl ex DC. (E 86)	Branches	+ +	+	−	−	−
COMPOSITAE						
Ambrosia ambrosioides (Cav.) Payne (E 111)	Branches	+ +	+	−	−	+
Ambrosia psilostachya DC. (E 21)	Whole plant	+ +	+ +	+ +	−	−
Anaphalis margaritacea (L.) A. Gray (E 17)	Branches	+	+ +	−	−	−
Aster spinosus Benth. (E 14)	Whole plant	−	−	−	−	−
Baccharis glutinosa Pers. (E 12)	Branches	+ + +	+ +	+ +	−	−
Bebbia atriplicifolia (A. Gray) Greene (E30)	Branches	+ +	+ +	+ +	−	−
Bidens pilosa L. var. *radiata* Schulz-Bip. (E 28)	Branches	+	−	+	−	−
Haplopappus sonoriensis (A. Gray) S.F. Blake (E 46)	Branches	+ +	+ +	+	−	−
Hymenoclea monogyra Torr. and Gray (E 47)	Branches	+ +	+ + +	+ +	−	−
Perityle microglossa Benth. (E 66)	Whole plant	+ +	+ + + +	−	−	−
Porophyllum gracile Benth. (E 25)	Branches	−	−	−	−	−
Sonchus oleraceus L. (E 92)	Branches	−	−	−	−	−

Calliandra cf. *californica* Benth. (E 94)	Whole plant	+ +	+ +	–	–	+ +
Calliandra penisularis Rose (E 127)	Branches	+	–	–	–	+ +
Pithecellobium confine Standley (E 104)	Fruit	+	–	–	–	+ + +
Pithecellobium dulce (Roxb.) Benth. (E 99)	Branches	+ + +	–	–	–	–
LOGANIACEAE						
Buddleia crotonoides A. Gray (E 98)	Branches	–	–	–	–	–
MALPIGHIACEAE						
Mascagnia macroptera (Sessé) and Moc.) Niedenzu (E 108)	Branches	+ +	+ +	–	–	+ +
OLEACEAE						
Fraxinus uhlei (Wenz.) Ling. (E 83)	Branches	+ + +	+	–	–	–
ONAGRACEAE						
Ludwigia octovalvis (Jacq.) Raven (E 15)	Branches	+ + +	–	–	–	+ + + +
PASSIFLORACEAE						
Passiflora edulis Sims (E 102)	Branches	+ +	+	–	–	–
PHYTOLACCACEAE						
Stegnosperma halimifolium Benth. (E 33)	Branches	–	+	–	–	–
POLEMONIACEAE						
Loeselia ciliata L. (E 39)	Whole plant	+	–	+	–	–

TABLE 1 (*continued*)

Family Botanical name (Voucher specimen)	Plant part tested	Antibacterial activity[a] A[b]	B	C	D	E
POLYPODIACEAE						
Pellaea ternifolia (Cav.) Link var. *ternifolia* (E 13)	Whole plant	+ + +	+	−	−	−
Thelypteris puberula (Baker) Morton var. *sonorensis* A. Reid Smith (E 20)	Whole plant	+	−	−	−	+
POLYGONACEAE						
Antigonon leptopus Hook. and Arn. (E 45)	Root	−	−	−	−	−
Polygonum lapathifolium L. (E 16)	Whole plant	+ +	−	−	−	−
PORTULACACEAE						
Talinum paniculatum (Jacq.) Gaertn. (E 37)	Root	−	−	−	−	−
RHAMNACEAE						
Colubrina glomerata (Benth). Hemsl. (E 8)	Branches	−	−	−	+	−
Karwinskia humboldtiana (Roem. and Schult.) Zucc. (E 4)	Branches	+ + +	+	−	−	−
RUTACEAE						
Citrus aurantifolia (Christm.) Swingle aff. (E 74)	Branches	−	−	−	−	−
Citrus paradisi Macf. (E 73)	Branches	+	+	−	−	−

SAURURACEAE						
Anemopsis californica (Nutt.) Hook. and Arn. (E 60)	Whole plant	–	–	–	–	–
SELAGINELLACEAE						
Selaginella lepidophylla (Hook. and Grev.) Spring. (E 5)	Whole plant	–	–	–	–	–
SOLANACEAE						
Datura discolor Bernh. (E 42)	Branches	+ +	+	+ +	–	+
Nicotiana glauca R. Graham (E 10)	Leaves	–	–	–	–	–
Solanum erianthum D. Don (E 48)	Branches	+ +	–	–	–	–
Solanum hindsianum Benth. (E 32)	Branches	+ +	+ +	–	–	+
Solanum nigrum L. (E 71)	Branches	+ + +	+ +	–	–	–
STERCULIACEAE						
Melochia tomentosa L. (E 76)	Branches	–	–	–	–	–
Waltheria americana L. (E 103)	Branches	+ +	–	–	–	–
TURNERACEAE						
Turnera diffusa Willd. (E 43)	Branches	+	+ +	–	–	–
UMBELLIFERAE						
Arracacia brandegeei Coulter and Rose (E 18)	Root	+ +	+	–	–	–
URTICACEAE						
Pilea microphylla Liebm. (E 78)	Branches	–	–	–	–	–

TABLE 1 (*continued*)

Family Botanical name (Voucher specimen)	Plant part tested	Antibacterial activity[a] A[b]	B	C	D	E
VERBENACEAE						
Lantana camara L. (E 69)	Branches	+ + +	+ + +	+ + + +	−	−
Lantana glandulosissima Hayek (E 6)	Branches	−	−	−	−	−
Lippia palmeri S. Wats. var. *palmeri* (E 44)	Branches	+ +	+	+ +	+	+ +
Lippia formosa Brandegee (E 22)	Branches	+ + + +	+ + +	+ +	−	−
ZYGOPHYLLACEAE						
Larrea tridentata Sessé and Moc. ex DC. (E 112)	Branches	+ + +	+ + +	+ + +	−	−

[a]Grading of results: −, no zone of inhibition; +, zone of inhibition less than 10 mm in diameter; + +, zones of inhibition of 10−15 mm in diameter; + + +, zone of inhibition 15−20 mm in diameter; + + + +, zone of inhibition more than 20 mm in diameter.
[b]Bacterial strains: A, *Staphylococcus aureus*; B, *Bacillus subtilis*; C, *Streptococcus faecalis*; D, *Escherichia coli*; E, *Candida albicans*.
[c]Voucher specimen: E 40 = Encarnación 40.

ternifolia (Polypodiaceae), *Karwinskia humboldtiana* (Rhamnaceae), *Solanum nigrum* (Solanaceae), *Lantana camara* and *Lippia formosa* (Verbenaceae), and *Larrea tridentata* (Zygophyllaceae).

(2) The most active extracts against *Bacillus subtilis* are from *Aristolochia brevipes* (Aristolochiaceae), *Parkinsonia aculeata* (Caesalpiniaceae), *Hymenoclea monogyra* and *Perityle microglossa* (Compositae), *Lantana camara* and *Lippia formosa* (Verbenaceae) and *Larrea tridentata* (Zygophyllaceae).

(3) The most active extracts against *Streptococcus faecallis* are *Lepechinia hastata* (Labiatae), *Lantana camara* (Verbanaceae) and *Larrea tridentata* (Zygophyllaceae).

(4) Slight activity against *E. coli* was shown by extracts from *Wislizenia refracta* (Capparidaceae), *Colubrina glomerata* (Rhamnaceae) and *Lippia palmeri* (Verbenaceae).

(5) The most active extracts against *C. albicans* are from *Pithecellobium confine* (Mimosaceae) and *Ludwigia octovalvis* (Onagraceae).

(6) Extracts which showed slight activity against Gram-positive and Gram-negative microorganisms are from *Wislizenia refracta* (Capparidaceae) whereas those showing slight activity against Gram-positive microorganisms and *C. albicans* are from *Bursera microphylla* (Burseraceae), *Ambrosia ambrosioides* (Compositae), *Euphorbia* cf. *polycarpa* (Euphorbiaceae), *Fouquieria diguettii* (Fouquieriaceae), *Calliandra* cf. *californica* and *Calliandra peninsularis* (Mimosaceae), *Mascagnia macroptera* (Malpighiaceae), *Thelypteris puberula* (Polypodiaceae), and *Datura discolor* and *Solanum hindsianum* (Solanaceae).

Discussion and Conclusions

If we compare the uses of the plants in Table 1 as previously reported in traditional medicine (Encarnación and Agundez, 1986; and Encarnación et al., 1987) against the test results, it can be seen that 29 (40%) extracts are very active or slightly active against one or several of the microorganisms used in this bioassay. From this group of plants, only *Wislizenia refracta* (Capparidiaceae), *Ludwigia octovalvis* (Onagraceae), *Pellaea ternifolia* (Polypodiaceae) and *Fouquieria diguetti* (Fouquieriaceae) have no record of use in traditional medicine against any kind of infectious diseases. Other plants have been reported to be used against one or more of the following complaints: constipation, infected wounds, pimples, kidney pain, cold sinusitis, toothache, fever, bronchitis, stomachache, cistitis, urethritis and venereal diseases, sicknesses that in some way could be produced by pathogenic microorganisms. These results clearly show a good correlation between traditional medicine and the antimicrobial screening results. These results of the present study serve as a guide which may help us select plants with antimicrobial activity for further work on the isolation and elucidation of the active compounds.

Based on the test data, further chemical and pharmacological investiga-

tions may be recommended for *Lippia palmeri* (Verbenaceae), because of its activity against all the test organisms, including *C. albicans*. Additionally, for *Lepechinia hastata* (Labiatae), active against three test organisms, we have isolated carnosol from this plant as one of the active compounds present in the chloroform crude extract. The results of this investigation will be communicated as a separate paper.

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