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Antimicrobial activity of selected Peruvian medicinal plants

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Abstract

The antimicrobial activity of 36 ethanol extracts from 24 plants, all of them currently used in the Peruvian traditional medicine for the treatment of several infectious and inflammatory disorders, was tested by means of the agar-well diffusion assay against four bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and four fungi (*Candida albicans*, *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Sporothrix schenckii*). Twenty-five (69%) extracts showed some degree of antimicrobial activity against at least one microorganism. The plants with the greatest antimicrobial activity were *Cestrum auriculatum* L. Heritier (Solanaceae), *Iryanthera lancifolia* Ducke Suesseng (Myristicaceae), *Lepechinia meyenii* (Walp.) Epling (Lamiaceae) and *Ophryosporus peruvianus* (Gmelin) King & H. Rob. (Asteraceae).

Keywords: Agar-well diffusion assay; Antimicrobial; Medicinal plants; Perū

1. Introduction

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use.

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms (Harborne, 1990). These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher et al., 1987). There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of them to yield active principles (Rabe and van Staden, 2000; Palombo and Semple, 2001; Portillo et al., 2001; Srinivasan et al., 2001; El-Seedi et al., 2002; Zgoda-Pols et al., 2002).

From an estimated 250,000 higher plants in the world (Wilson, 1988), only 5–15% have been studied for a potential therapeutic value (Balandrin et al., 1985; Kinghorn, 1992). A large number remains to be investigated.

In Perū, like in other developing countries, medicinal plants still represent the main therapeutic tool in traditional medicine. The Peruvian flora offers great possibilities for the discovery of new compounds with antimicrobial activity. It is estimated that 17,144 flowering plant species occur in Perū of which 5354 (31.3%) are endemic (Brack, 1999).

The main objective of this study is to search for Peruvian medicinal plants with strong antimicrobial activity which could serve as good candidates for the development of new antimicrobial agents and/or standardized phytomedicines.

In this paper, we report the results of study for antimicrobial activity of 24 plants used in the Peruvian traditional medicine for the treatment of several infectious or inflammatory diseases. The plants were chosen based on their reported uses in the literature (Brack, 1999; Duke and Vasquez, 1994;

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Rutter, 1990) and on ethnobotanical surveys conducted in three different regions of Perū.

2. Material and methods

2.1. Plant material

Ethnobotanical surveys were conducted in three different regions of Perū by the botanists Irma Fernández (IF) and Joaquina Albán (JA) between May and July 2001 (Table 1). Plants were selected based on their traditional use for the treatment of gastrointestinal, genitourinary, respiratory and skin infections. The data were collected by performing interviews with the traditional healers ("curanderos") and with the native people of the villages. Before starting the ethnobotanical surveys, the botanists (IF, JA) obtained written consents from the local authorities of all collection sites. All the interviewees were informed on the scope and goals of the project and on the potential publication of the results of the study. A review of the literature was performed along with the ethnobotanical surveys (Brack, 1999; Duke and Vasquez, 1994; Rutter, 1990). Plants were collected and identified by IF and JA. The respective voucher specimens have been deposited at the Department of Chemistry of the Pontificia Universidad Católica del Perú, in Lima.

2.2. Preparation of extracts

Air-dried powdered plant material was extracted by percolation at room temperature with 95% ethanol. The solvent was then evaporated to dryness under reduced pressure at a temperature lower than 40 °C. Yields of extracts in terms of dry starting materials are listed in Table 2. For the bioassay, the extracts were resuspended in 95% ethanol at a concentration of 25 mg/ml.

2.3. Microorganisms

The bacteria were obtained from the Laboratorio de Microbiología of the Universidad Peruana Cayetano Heredia (MBCH, Lima, Perū). Two Gram-positive (*Bacillus subtilis* MBCH 012 and *Staphylococcus aureus* MBCH 021) and two Gram-negative (*Eschrerichia coli* MBCH 016 and *Pseudomonas aeruginosa* MBCH 009) bacteria were used for the study. Identification and maintenance of cultures were performed by one of us (JB) using classical diagnostic microbiology procedures (Finegold and Martin., 1982).

The yeast *Candida albicans* ATCC 90028 was obtained from the American Type Culture Collection (ATCC, Rockville, MD), while *Trichophyton mentagrophytes* var. *interdigitale* IHEM 0584 was provided by the Belgian Coordinated Collection of Microorganisms (BCCM/IHEM, Brussels, Belgium). The other species of fungi were clinical isolates obtained from the Laboratorio de Micología of the Instituto de Medicina Tropical "Alexander von Humboldt" (IMTAvH, Lima, Perū). They belong to filamentous fungus *Microsporum gypseum* IMTAvH 18051 and dimorphic fungus *Sporothrix schenckii* IMTAvH 36836. *Microsporum gypseum* and *Sporothrix schenckii* were isolated from a patient with tinea corporis and a patient with disseminated cutaneous sporotrichosis, respectively. Both microorganisms were identified by one of us (BB) by classical microbiology techniques (de Hoog et al., 2000).

2.4. Antimicrobial activity

The antimicrobial activities of the ethanol extracts were evaluated by means of the agar-well diffusion assay. The assay was carried out according to the method of Hufford et al. (1975) with some modifications. The media used were Mueller–Hinton agar (Scharlau Chemie) for the bacteria and Sabouraud Dextrose agar (Difco) for the fungi. Twenty milliliters of the specified molten agar (45 °C) was aseptically mixed with either 100 μl of a bacterial suspension (3×10^8 CFU/ml) or 1 ml fungal suspension (1×10^4 CFU/ml) and poured into 100 mm × 15 mm sterile Petri dishes. (For the preparation of the inocula: colonies of bacteria and fungi were suspended in Mueller–Hinton broth and sterile saline, respectively. The suspensions were adjusted turbidimetrically to a McFarland 1 for bacteria, to 0.5 for *Candida albicans* and by using a hemacytometer cell counting chamber for the other fungi. The concentration of the suspensions were corroborated by serial dilution plate counts). Once the agar was hardened, 11 mm wells were bored using a sterile cork borer. A 0.1 ml of the ethanolic extracts (25 mg/ml) were placed into the wells and the plates were incubated for 24 h at 37 °C for the bacteria and 24–72 h at room temperature for the fungi. Oxacillin (1 μg/disk) and norfloxacin (10 μg/disk) (Difco, Detroit, MI) served as positive controls for Gram-positive and Gram-negative bacteria, respectively. Amphotericin B (0.2 mg/ml) (Sigma) and fluconazol (0.2 mg/ml) (Pfizer) were dissolved in DMSO (Sigma) and served as positive controls for the fungi. The tests were carried out in triplicate. The antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. Solvent controls (95% ethanol and DMSO) were included in every experiment as negative controls.

3. Results

Table 1 summarizes the ethnobotanical data of the plant species selected for the study. Thirty-six ethanol extracts from 24 plants (belonging to 16 different families) were tested for antimicrobial activity against four bacteria and four fungi using the agar-well diffusion assay. The results of this screening are presented in Table 2.

Twenty-five (69.4%) of the plant extracts showed some degree of activity against at least one of the microorganisms. The plants which exhibited significant antimicrobial activity (defined as a perfectly clear zone with a diameter greater

Table 1
Ethnobotanical data of the plants investigated

Latin binomial	Family	Common name	Voucher specimen #	Part used[a]	Collection site[b]	Popular uses[c]
Aloysia scorodonioides (Kunth) Cham.	Verbenaceae	Españolada	IF1544	AP	H	Treatment of diarrhea, vaginitis
Ambrosia arborescens Mill.	Asteraceae	Marco	IF1517	L, S	H	Antiseptic, anti-inflammatory, insect repellent
Aspidosperma rigidum Rusby	Apocynaceae	Remo caspi	JA13629	B, L, S	L	Treatment of diarrhea, malaria
Cestrum auriculatum L. Heritier	Solanaceae	Hierba santa	JA13602	L, S	P	Treatment of allergies, fever, diarrhea. Wound healing
Chuquiraga spinosa D. Don	Asteraceae	Huamanpinta	IF1515	AP	H	Anti-inflammatory, gonorrhoea
Croton ruizianus Muell-Arg.	Euphorbiaceae	Kasmanlle	JA13626	L, S	P	Wound healing, antiseptic
Cynanchum corystephanum Malm.	Asclepiadaceae	Aurinsha	IF1543	AP	H	Treatment of vaginitis, wound healing
Desmodium molliculum (Kunth) DC.	Fabaceae	Manayupa	IF1529	AP	H	Wound healing, anti-inflammatory, antiseptic
Euterpe precatoria Mart.	Arecaceae	Chonta	JA13630	R	L	Treatment of hepatitis, dysmenorrhea, diarrhea
Flaveria bidentis (L.) Kuntze	Asteraceae	Matagusano	IF1518	AP	H	Treatment of coughs and intestinal parasites. Antiseptic
Iochroma umbellatum (R. & P.) Hunziker ex D'Arcy	Solanaceae	San Pablo	JA13624	L, S	P	Wound healing, antiseptic
Iryanthera lancifolia Ducke Suesseng.	Myristicaceae	Cumalilla	JA13656	B, L, S	L	Treatment of diarrhea, fever. Antispasmodic
Jungia paniculata (DC.) A. Gray	Asteraceae	Caramate	IF1523	AP	H	Wound healing, antiseptic, anti-inflammatory
Justicia sericea R. & P.	Acanthaceae	Arzobispo	IF1541	AP	H	Treatment of vaginitis, anti-inflammatory
Lavatera arborea L.	Malvaceae	Malva	IF1542	L	H	Treatment of vaginitis, wound healing
Lepechinia meyenii (Walp.) Epling	Lamiaceae	Tecuar	IF1530	AP	H	Treatment of coughs and diarrhea, antispasmodic
Oenothera multicaulis R. & P.	Onagraceae	Chupasangre	IF1533	WP	H	Wound healing, antiseptic
Ophryosporus peruvianus (Gmelin) King & H. Rob.	Asteraceae	Shekla	JA13591	L, S	P	Wound healing, antiseptic
Sambucus mexicana var. *bipinnata* (Schltdl. & Cham.) Schwer.	Caprifoliaceae	Sauquillo	JA13594	F, L, S	P	Treatment of cough, fever, urethritis, measles
Satureja elliptica (R. & P.) Briq.	Lamiaceae	Anchis	IF1525	AP	H	Treatment of coughs and flu
Senecio culcitioides Schultz-Bip.	Asteraceae	Ancosh	IF1531	AP	H	Treatment of coughs, asthma, respiratory diseases
Senecio violaefolius Cabrera	Asteraceae	Huamanripa	IF1532	AP	H	Treatment of coughs, asthma, respiratory diseases
Tetracera volubilis L. ssp. *volubilis*	Dilleniaceae	Paujil chaqui	JA13528	S, SB	L	Treatment of diarrhea and syphillis
Wigandia urens (R. & P.) Kunth	Hydrophyllaceae	Shinua	JA13592	WP	P	Treatment of cough, flu, bronchitis

[a] Plant part: AP, aerial parts; B, bark; F, flowers; L, leaves; R, roots; S, stems; WP, whole plant.
[b] Collection site: H, Huaraz, Department of Ancash; L, Yahuasyacu river, Department of Loreto; P, Pamparomas, Department of Ancash.
[c] Based on ethnobotanical surveys.

Table 2
Antimicrobial activities of crude ethanol extracts

Plant species	Plant parts tested[a]	Extract yield (%)[b]	Growth inhibition zone diameter (mm)							
			Antibacterial activities[c]				Antifungal activities[d]			
			B.s.	*S.a.*	*E.c.*	*P.a.*	*C.a.*	*T.m.*	*M.g.*	*S.s.*
Aloysia scorodonioides	AP	19.3	0	0	0	0	0	0	0	0
Ambrosia arborescens	L, S	14.4	0	0	0	0	0	0	0	0
Aspidosperma rigidum	B	32.7	19	0	0	0	0	0	0	0
	L	8	13	0	0	13	0	0	0	0
	S	0.7	17	0	0	13	0	0	0	0
Cestrum auriculatum	L	10.3	0	0	0	0	19	25	21	25
	S	5.9	0	0	0	0	0	0	0	0
Chuquiraga spinosa	AP	25.5	0	0	0	0	0	0	0	0
Croton ruizianus	L	13.4	13	13	13	13	13	0	0	0
	S	8.2	17	15	13	13	13	0	0	13
Cynanchum corystephanum	AP	17.5	0	0	0	0	0	0	0	0
Desmodium molliculum	AP	9.8	0	0	0	0	13	0	0	0
Euterpe precatoria	R	2.3	13	13	13	13	0	0	0	0
Flaveria bidentis	AP	44.8	0	0	0	0	0	0	0	0
Iochroma umbellatum	L	8.5	19	17	0	0	0	13	0	0
	S	6.4	13	13	0	0	0	0	0	0
Iryanthera lancifolia	L	10.3	15	15	0	0	13	13	0	0
	S	5.1	17	19	0	15	13	31	13	13
	B	12.9	15	13	0	0	13	0	0	0
Jungia paniculata	AP	8.3	0	0	0	0	0	0	0	0
Justicia sericea	AP	22.9	0	0	0	0	0	0	0	0
Lavatera arborea	L	11.8	0	0	0	0	0	0	0	0
Lepechinia meyenii	AP	11.9	23	23	13	21	17	15	0	0
Oenothera multicaulis	R	13.8	15	17	17	19	0	15	13	19
	AP	4.6	21	17	0	21	13	0	0	15
Ophryosporus peruvianus	L, S	4.3	0	0	0	0	17	27	0	13
Sambucus mexicana	L	17.1	0	0	0	0	0	0	0	0
	S	3.2	0	0	0	0	0	0	0	0
	F	6.7	0	0	13	0	0	0	0	0
Satureja elliptica	AP	10	0	0	13	0	0	0	0	0
Senecio culcitioides	AP	13.3	0	0	0	0	19	17	0	15
Senecio violaefolius	AP	27.8	13	0	0	0	0	13	0	0
Tetracera volubilis	S	13.1	17	17	0	15	0	0	0	0
	B	17.9	17	17	0	15	0	0	0	0
Wigandia urens	L	8.5	17	15	13	19	13	19	13	13
	S	4.4	15	15	13	13	13	0	0	13
Controls										
95% ethanol			0	0	0	0	0	0	0	0
DMSO			0	0	0	0	0	0	0	0
Oxacillin (1 μg/disk)			12	14						
Norfloxacin (10 μg/disk)					26	35				
Amphotericin B (0.2 mg/ml)							17	23	13	13
Fluconazol (0.2 mg/ml)							15	13	0	0

[a] Plant parts tested: AP, aerial parts; B, bark; F, flowers; L, leaves; R, roots; S, stems; WP, whole plant.
[b] Dry residue of the ethanolic extract in terms of dry extracting material.
[c] Bacterial species: *B.s.*, *Bacillus subtilis*; *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*.
[d] Fungi: *C.a.*, *Candida albicans*; *T.m.*, *Trichophyton mentagrophytes*; *M.g.*, *Microsporum gypseum*, *S.s.*, *Sporothrix schenckii*.

than 18 mm) were: *Aspidosperma rigidum*, *Cestrum auriculatum*, *Iochroma umbellatum*, *Iryanthera lancifolia*, *Lepechinia meyenii*, *Oenothera multicaulis*, *Ophryosporus peruvianus*, *Senecio culcitioides* and *Wigandia urens*. The most susceptible microorganisms were *Bacillus subtilis* and *Trichophyton mentagrophytes*, which were strongly inhibited by four (11%) of the extracts. On the other hand, none of the extracts inhibited *Escherichia coli*. The greatest zone of inhibition (31 mm) was displayed by *Iryanthera lancifolia* stem extracts against the dermatophytic fungus *Trichophyton mentagrophytes*.

The solvents (95% ethanol and DMSO) used for dissolving the crude extracts and the standard antibiotics always gave negative results, showing that they did not influence in the antimicrobial activities observed for the plant extracts (Table 2).

Table 3
Biological and phytochemical studies reported for the most active plants

Botanical species	Plant part	Biological activity	Compounds that have been isolated
Cestrum auriculatum	Leaves	Antibacterial activity (Neto et al., 2002)	None
Iryanthera lancifolia	Stem bark	antioxidant (Lee et al., 1998)	None
	Pericarp	None	Aryltetralinic lignan, a pair of epimeric 2-alkenyl-γ-lactones and one tocotrienol (Lopes et al., 1998)
	Pericarp	Antioxidant (Silva et al., 1999)	Dihydrochalcones and flavonolignans (Silva et al., 1999)
Lepechinia meyenii	Leaves	None	Guaiol (Mango et al., 1990)
	Aerial parts	None	Pisiferol, rosmanol, carnosic acid, salvicanol, isosalvicanol, 12-formyl-11-hydroxy-8,11,13-abietantrien-2-oic acid methyl ester (Bruno et al., 1991)
Ophryosporus peruvianus	Aerial parts	None	3,5-bis-Isovaleryl-*p*-hydroxyacetophenone (Bohlmann et al., 1984)

4. Discussion

The leaves of *Cestrum auriculatum* ("hierba santa", "hierba santa hembra", "hierba hedionda") are used by the community of Pamparomas for the treatment of skin infections and allergies. The leaves are usually rubbed with water and the extract obtained is applied directly to the skin. This water extract is also taken orally in small quantities for the treatment of fever and diarrhea. Other popular uses found in the literature for *Cestrum auriculatum* are for the treatment of hemorrhoids and headaches (Hammond et al., 1998) and as an antirheumatic and astringent (De Feo, 1992).

The filamentous fungus *Microsporum gypseum* and *Sporothrix schenckii* were inhibited by the ethanol extract of the leaves of *Cestrum auriculatum*. This extract showed no antibacterial activity, but demonstrated strong selective antifungal activity. Neto et al. (2002) demonstrated that *Cestrum auriculatum* leaf extracts was active against several Gram-positive and Gram-negative bacteria, however, as shown in this study, it was inactive against *Staphylococcus aureus* and *Escherichia coli*. No phytochemical investigation has previously been performed on this plant.

Iryanthera lancifolia and *Ophryosporus peruvianus* proved to be the most effective in selectively inhibiting *Trichophyton mentagrophytes*. There are no previous reports on the antimicrobial activity of these two species. However, an antioxidant activity in vitro has been demonstrated for *Iryanthera lancifolia* (Table 3). *Lepechinia meyenii* showed mainly broad antibacterial activity (Table 2) and several compounds have been previously isolated from this plant (Table 3).

Sporotrichosis is a chronic cutaneous and subcutaneous disease caused by the dimorphic fungus *Sporothrix schenckii* and is considered to be hyperendemic in the south central highlands of Perū (Bustamante and Campos, 2001). Untreated sporotrichosis can lead to scarring and disfigurement. Standard therapy for this disease relies mainly on itraconazole, however, potassium iodide is the drug of choice in developing countries like Perū because it is less expensive than itraconazole (Pappas et al., 2000). It is interesting that 5 (9%) of the extracts inhibited strongly the growth of *Sporothrix schenckii*, which was not susceptible to the standard antifungal agents amphotericin B and fluconazol. The fungus was more susceptible to the leaf extracts of *Cestrum auriculatum*. Bioassay-guided isolation studies on this plant could provide useful leads for the treatment of sporotrichosis.

5. Conclusions

Antimicrobial study of Peruvian plants, all of them selected based on their relevant ethnomedical use, has provided various extracts with strong activity against several pathogenic microorganisms. The results of this study support the folkloric use of many of these plants. The plants with the greatest antimicrobial activity were *Cestrum auriculatum*, *Iryanthera lancifolia*, *Lepechinia meyenii* and *Ophryosporus peruvianus*. It is still unknown which compounds are responsible for their biological activity; bioassay-guided isolation and identification of the active principles of these plants are now in progress.

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