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RESEARCH ARTICLE

Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*

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Abstract

Context: Subcutaneous mycoses are chronic infections caused by slow growing environmental fungi. Latin American plants are used in folk medicine to treat these afflictions. Moreover, the potential of the rich Latin American biodiversity for this purpose has not been fully explored.

Objectives: The aim of the study was to screen Latin American plant extracts against two species of subcutaneous fungi: *Sporothrix schenckii* and *Fonsecaea pedrosoi*.

Materials and methods: One hundred ninety-five organic extracts from 151 Latin American plants were screened against two subcutaneous fungi by the agar dilution method at a concentration of 100 µg/mL, and minimum inhibitory concentrations (MICs) of active extracts were determined. Positive (amphothericin B) and negative (50% ethanol) controls were used.

Results and discussion: Twenty eight extracts showed activity at ≤100 µg/mL. Of these, four extracts from *Gnaphalium gaudichaudianum* DC (Asteraceae), *Plumeria rubra* L (Apocynaceae), *Tecoma stans* (L.) Juss. ex Kunth. (Bignoniaceae), and *Trichostigma octandum* (L.), H. Walter showed activity against *F. pedrosoi* at MIC 12.5 µg/mL; and, four extracts from *Bourreria huanita* (Lex.) Hemsl. (Boraginaceae), *Phytolacca bogotensis* Kunth (Phytolaccaceae), *Monnina xalapensis* Kunth (Polygalaceae) and *Crataegus pubescens* (C. Presl) C. Presl (Rosaceae) against *S. schenckii*. This is the first report on antifungal activity of the Latin American plants against these two subcutaneous fungi.

Conclusion: *S. schenkii* and *F. pedrosoi* were inhibited by *B. huanita* (MIC: 12.5 and 25 µg/mL), *G. gaudichaudianum* (MIC: 50 and 12.5 µg/mL) and *T. triflora* (MIC: 25 µg/mL).

Keywords: *Bourreria huanita*, *Fonsecaea pedrosoi*, Gnaphalium gaudichaudianum, *Sporothix schenkii*, Terminalia triflora

Introduction

Sporotrichosis is a subcutaneous fungal disease, usually associated with trauma. It is caused by the dimorphic fungus *Sporothrix schenckii*. It occurs globally in tropical and subtropical regions and is characterized by nodular lesions, associated with lymphadenopathy (Morris-Jones, 2002; Ramos-e-Silva et al., 2007), which become disseminated in AIDS patients (Ware et al., 1999). The available therapy in the majority of patients consists of treatment with terbinafine (Hull & Vismer, 1992), potassium iodide (Sandhu & Gupta, 2003), amphotericin B (Kohler et al., 2007), itraconazole and ketoconazole (Alvarado-Ramírez & Torres-Rodríguez, 2007).

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Chromoblastomycosis is a traumatically induced chronic subcutaneous mycotic infection, which is caused by several species of pigmented saprophytic molds. Among them, *Fonsecaea pedrosoi* is one of the most common pathogens associated with this disease, which progresses slowly over many years. The currently available drugs 5-flucytosine, fluconazole, ketoconazole, amphotericin B (Lupi et al., 2005; López Martínez & Méndez Tovar, 2007), itraconazole and terbinafine (Gupta et al., 2002) show toxicity, limited efficacy, and require longer duration of treatments. This results in the development of resistance as reported for itraconazole (Andrade et al., 2003). Therefore, there is an urgent need to discover newer antifungal drugs for subcutaneous mycoses.

Plants constitute an invaluable source of antifungal compounds because of their unmatched availability of chemical diversity, and many efforts have been made worldwide to identify medicinal plants with antifungal properties (Rai & Mares, 2003; Cos et al., 2006; Maregesi et al., 2008), some of them in Latin America (Agüero et al., 2007; Cruz et al., 2007; Danelutte et al., 2003; Escalante et al., 2002; Malheiros et al., 2005, Svetaz et al., 2010).

There have been a few studies reported on antifungal screening of medicinal plants against subcutaneous fungi. For example, against *S. schenckii*, Rojas et al. (2003) reported results of antifungal screening of 24 Peruvian plants; the essential oil of *Origanum vulgare* L. (Lamiaceae) showed activity against seven isolates with a MIC of 250 µL/mL (Cleff et al., 2008); the ethanol extract of *Agapanthus africanus* (L.) Hoffmanns (Agapanthaceae) rhizomes showed activity at a MIC of 250 µg/mL, and a saponin was isolated and active at MIC: 15.6 µg/mL (Singh et al., 2008); and, pseudodillapiol, isolated from *Piper abutiloides* Kunth (Piperaceae), showed activity at 12.5 µg/mL (Johann et al., 2008).

On the other hand, four studies on plants acting against *F. pedrosoi* showed moderate activity (MIC 200–625 µg/mL) in Brazil, on essential oil of *Lippia origanoides* Kunth. (Verbenaceae) (Oliveira et al., 2007), aqueous extract of *Caesalpinia pyramidalis* Tul. (Caesalpinaceae) and *Ziziphus joazeiro* Mart. (Rhamnaceae) (Cruz et al., 2007), seven *Artemisia* species (Lopes-Lutz et al., 2008), and methanol extract from *Pterocaulon alopecuroides* Lam (Asteraceae) (Daboit et al., 2009).

The only study that investigated both fungi is one on the defatted ethanol extract of *Curcuma longa* L. (Zingiberaceae) conducted in Thailand, which showed inhibition of *S. schenckii* (MIC 114 µg/mL) and *F. pedrosoi* (459 µg/mL), although the isolated curcumin did not present activity against neither (Apisariyakul et al., 1995).

Within the framework of a multinational collaborative Organization of American States (OAS) project coordinated by one of the authors (MPG) during 2001–2004 with the participation of research centers from Argentina, Bolivia, Brazil, Colombia, Costa Rica, Guatemala and Panama, the potential of 327 plant species (92 families and 251 genera) from Latin America were screened for antifungal properties against a panel of human opportunistic fungi (Svetaz et al., 2010).

We now report here the antifungal activity of 151 species from Latin America used either ethnomedically for skin or dermatomucosal diseases or collected at random in 0.1 h biodiversity plots in protected reserves against *S. schenckii* and *F. pedrosoi*, the two most prevalent agents of subcutaneous infections.

Materials and methods

Plant material

One hundred fifty-one plants from 64 families from seven countries involved in the OAS project were chosen and collected (Table 1). These included 112 species used in ethnomedicine for the following conditions of traditional therapeutic indications: antibiotic, antifungal, antiseptic, infections, itching, respiratory diseases, skin ailments, ulcerations, vaginitis, venereal diseases, vulnerary, and wound healing, and 39 species collected at random from 0.1 ha biodiversity plants in protected reserves.

The taxonomic identity of each plant was established by taxonomists of participating countries from Argentina, Elisa Petenatti and Martha Gattuso; from Bolivia, Rosy de Michel, Genevieve Bourdy and Andrés Roca; from Colombia, Ricardo Callejas, Edgar Linares, Zaleth Cordero, and Santiago Díaz; from Costa Rica, Luis Guillermo Acosta, Alexander Rodríguez and Diego Vargas; from Guatemala, Mario Véliz and Juan José Castillo; and, from Panama, Mireya Correa.

Voucher specimens were deposited in each country's Herbarium. Argentina: National University of San Luis (UNSL), San Luis: National University of Rosario (UNR), Rosario: National University of Entre Ríos (ERA), Paraná: and the Herbarium of the Botanical Institute of North East (IBONE). Bolivia: National Herbarium of Bolivia (LPB), La Paz, Brazil: Herbário Barbosa Rodrigues (HBR), Itajaí, SC. Colombia: National Colombian Herbarium (COL), Bogotá. Costa Rica: National Institute for Biodiversity, INBio (INB), San José. Guatemala: CEMAT-FARMAYA Ethnobotany Herbarium (CFEH), Guatemala. Panamá: FLORPAN at the Herbarium of the University of Panamá (PMA), Panamá (Table 1).

Preparation of extracts

From 151 species, 195 extracts were prepared by percolation with different solvents; percolates were concentrated *in vacuo* in a rotary evaporator at a temperature <40°C and dried in a vacuum dryer.

Antifungal assay

Activity was evaluated by the procedure described by Brancato and Golding (1983) for filamentous fungi, modified by Mac Rae et al. (1988) and previously used to determine activity against dermatophytes (Cáceres et al., 1993). Due to its marked survival in the environment, only the saprophytic phase was studied. Screw cap tubes containing Takashio agar slant for sporulation

Table 1. Antifungal activity against *Sporothrix schenckii* and *Fonsecaea pedrosoi* of extracts from Latin American plant species (MIC μg/mL).

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC μg/mL	
							Ss	Fp
Acanthaceae								
Blechum pyramidatum (Lam.) Urb.	Ncn*	P	Canal Area, La Kevie	FLORPAN 5343	Wh	EtOH	<100	<100
Justicia gilliesii (Nees) Benth	Albahaca del campo	A	San Luis Capital	UNSL Del Vitto & Petenatti 4281	L/F	MeOH	>100	>100
Justicia lindeniana Nees. J.F. Macbr.	Rax Pin	G	Sebolito, Chahal, AV	CFEH 850	L	EtOH	>100	>100
Justicia magniflora (S.F.Blake) D.N.Gibson	Numay pim	G	Sebolito, Chahal, AV	CFEH 851	L	EtOH	>100	>100
Justicia tweediana (Ness) Griseb.	Alfalfilla	A	San Luis Province	UNSL Del Vitto & Petenatti 4947	L/F	MeOH	>100	>100
Ruellia biolleyi Lindau	Ncn	P	Colón, Guanche river	FLORPAN 5920	Wh	EtOH	>100	>100
Amaryllidaceae								
Crinum erubescens Aiton	Lirio	CR	Carara Nat. Park	INB LA 2471	L/F	EtOH	>100	>100
Crinum darienense Woodson	Ncn	P	Camino de Cruces NP	FLORPAN 5678	F	EtOH	<100	<100
Anacardiaceae								
Schinus areira L.	Ncn	A	Mendoza, La Paz, Desaguadero	UNSL Del Vitto & Petenatti 3844	L/F	MeOH	100	>100
Apocynaceae								
Plumeria rubra L.	Palo de la Cruz	G	Samayac, Such.	CFEH 956	L	EtOH	>100	12.5
Rauvolfia tetraphylla L.	Chalchupa	G	Samayac, Such.	CFEH 437	Ro	EtOH	>100	>100
Arecaceae								
Socratea exorrhiza (Mart.) H. Wendl.	Ji	B	Sta, Rosa, La Paz	LPB SD 247	Ro	EtOH	>100	>100
Aristolochiaceae								
Aristolochia gibertii Hook.	Patito	A	El Palmar Nat. Park	ERA 25	L/F	MeOH	50	>100
Tessaria integrifolia Ruiz & Pav.	Cahuara	B	Abel Iturralde, La Paz	LPB SD 25	L	CH_2Cl_2	>100	>100
Asteraceae								
Baccharis medullosa DC	Ncn	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 9236	L/F	MeOH	>100	100
Baccharis notosergila Griseb.	Tipishá í.	A	Arminda, Rosario	UNR 649	L/F	MeOH	100	>100
Bidens subalternans DC	Amor seco	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 6580	L/F	MeOH	>100	>100
Cirsium subcoriaceum (Less.) Sch.Bip.	Ncn	CR	Los Santos Forest Res.	INB LDV 0774	A	EtOH	>100	>100
Dahlia imperialis Roetz-ex Ortgies	Ncn	C	Bogotá Savannah	COL 498592	L	EtOH	>100	>100
Flaveria bidentis (L.) Kuntze	Escoba amarilla	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 9236	L/F	EtOH	>100	>100
Gaillardia megapotamica (Spreng.) Baker, var. *megapotamica*	Topasaire, botón de oro	A	San Luis, Pringles, Carolina	UNSL Del Vitto & Petenatti 5825	L/F	MeOH	>100	100
Gnaphalium gaudichaudianum DC	Marcelita	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 9239	A	MeOH	50	12.5
Gochnatia glutinosa (Don) Don ex Hook. & Arn	Jarililla	A	Ayacucho, Belgrano, San Luis	UNSL Del Vitto & Petenatti 7461	L/F	EtOH	100	>100
Heterothalamus alienus (Spreng.) Kuntze	Romerillo	A	San Luis, San Martín, La Huertita	UNSL Del Vitto & Petenatti 6669	L/F	MeOH	>100	>100
Mikania periplocifolia Hook. & Arn.	Clavel de campo	A	San Luis, Belgrano, San Francisco	UNSL Del Vitto & Petenatti 2217	L/F	EtOH	>100	>100
Polymnia maculata Cav.	Ax	G	Coban, AV	CFEH 259	L	EtOH	50	>100
Senecio salignus DC.	Chilca	G	Parramos, Chimalt.	CFEH 437	L	EtOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL	
							Ss	Fp
Senecio subulatus D. Don ex Hook. & Arn.	Monteamargo	A	San Luis Mountain Chains	UNSL Del Vitto & Petenatti 9241	L/F	MeOH	>100	>100
Schistocarpha eupatorioides (Fenzl.) Kuntze	Ncn	C	Bogota Savannah	COL 498591	L	EtOH	>100	>100
Schkuhria pinnata (Lam.) Kuntze ex Thell.	Matapulgas canchalagua	A	Pringles, Río Grande, San Luis	UNSL Del Vitto & Petenatti 1766	Wh	EtOH	>100	>100
Sphagneticola trilobata (L.) Pruski	Ncn	P	Colón, Costa Arriba, Cuango	PMA FLORPAN 5904	L	EtOH	<100	<100
Solidago chilensis Meyen	Vara amarilla	A	San Luis, Junín, Merlo	UNSL Del Vitto & Petenatti 8600	L	MeOH	>100	>100
					F	MeOH	>100	>100
Tagetes filifolia Lag.	Anisillo	A	Pringles, Río Grande, San Luis	UNSL Del Vitto & Petenatti 5326	L	MeOH	>100	>100
Tithonia diversifolia Hemsl. (A. Gray)	Mirasol	G	Samayac, Such.	CFEH 684	L	EtOH	>100	>100
Bignoniaceae								
Clytostoma callistegioides (Cham.) Bureau ex Griseb.	Ncn	B	Cordillera, Sta. Cruz	LPB AR 16558	B	EtOH	>100	>100
Godmania aesculifolia (Kunth) Standl.	Corteza de chivo	CR	Lomas de Barbudal Biol. Reserve	INB LA 2583	Ro	EtOH	100	>100
Jacaranda mimosifolia D. Don	Jacarandá	A	San Luis, Capital	UNSL Del Vitto & Petenatti 9371	L	MeOH	>100	>100
Tabebuia rosea (Bertol.) A. DC.	Matilisguate	G	Jalapa	CFEH 343	L	EtOH	>100	>100
Tecoma stans (L.) Juss. ex Kunth	Timboco	G	El Progreso	CFEH 435	L	EtOH	100	>100
					B	EtOH	100	12.5
Tynanthus guatemalensis Donn. Smith	Bejuco de agua	G	La Libertad, Petén	CFEH 925	V	EtOH	>100	>100
Boraginaceae								
Bourreria huanita (Lex.) Hemsl.	Esquisuchil	G	Antigua, Sacatepequez	CFEH 895	F	EtOH	12.5	25
					L	EtOH	>100	>100
Cordia cylindrostachya (Ruiz & Pav.) Roem. & Schult	Leño negro	C	Cundinamarca	COL 498048	L	EtOH	>100	>100
Cordia gigante Poir.	Upay	G	El Jícaro, El Progreso	CFEH 963	L	EtOH	>100	>100
Ehretia cortesia Gottschling	Campa, cambao	A	Ayacucho, Sierra de las Quijadas, San Luis	UNSL Del Vitto & Petenatti 7911	L	MeOH	>100	>100
Tournefortia bicolor Sw.	Ncn	P	Altos de Campana Nat. Park	FLORPAN 5896	L	EtOH	<100	<100
Bromeliaceae								
Aechmea distichantha Lem.	Cardo chuzo	A	Sta. Fe, Las Gamas	UNR NC 2303	L/F	MeOH	>100	100
Bromelia plumieri (E. Morren) L.B. Smith	Piñuela, puya	CR	Lomas de Bardual Biol. Reserve	LA 3470	L	EtOH	>100	>100
Burseraceae								
Tetragastris panamensis (Engl.) Kuntze	Ncn	P	Cerro Ancón	FLORPAN 6531	L	EtOH	>100	>100
					S	EtOH	>100	>100
					Fr	EtOH	>100	>100
Caricaceae								
Jacaratia spinosa (Aubl.) A. DC.	Pajajaja	B	Abel Iturralde, La Paz	LPB AS 151	B	EtOH	>100	>100
Celastraceae								
Maytenus vitis-idaea Griseb.	Tala salado	A	Margarita, Santa Fe	UNR 1407	L	MeOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL Ss	MIC µg/mL Fp
Celastomataceae								
Gymnosporia urbaniana (Loes.) Liesner	Ncn	B	Abel Iturralde, La Paz	LPB GB1913	L	CH_2Cl_2	>100	>100
Celtidaceae								
Celtis pallida Torr.	Granjeno	A	San Luis, Junín, Merlo	UNSL Del Vitto & Petenatti 3493	L	MeOH	>100	>100
Chrysobalanaceae								
Licania arborea Seem.	D'reu	B	La Paz department	LPB GB 1790	L	CH_2Cl_2	>100	>100
Clusiaceae								
Chrysochlamys eclipes L.O. Williams	Ncn	P	Altos de Campana Nat. Park, Zamora	FLORPAN 4347	L	EtOH	>100	>100
					S	EtOH	>100	>100
Hypericum uliginosum Kunth	Retij	G	Tajumulco, SM	CFEH 970	L	EtOH	>100	>100
Rheedia gardneriana Planch & Triana	Jashau	B	Abel Iturralde, La Paz	LPB SD 110	L	CH_2Cl_2	>100	>100
Combretaceae								
Combretum laxum Jacq.	Bejuco guayaba	A	Iguazú Nat. Park	IBONE 35249	L/F	MeOH	100	>100
					L	MeOH	100	>100
Terminalia australis Camb.	Palo amarillo	A	Entre Ríos	ERA 23	L	MeOH	100	>100
					L/F	MeOH	>100	>100
					L	EtOH	100	>100
					L	H_2O	100	>100
Terminalia triflora (Griseb.) Lillo	Palo amarillo	A	Entre Ríos	ERA 24	L	EtOH	25	25
Cornaceae								
Cornus peruviana L.	Lloró	CR	Braulio Carrrillo Nat. Park	LDV 0777	L	EtOH	>100	>100
Cucurbitaceae								
Psiguria warscewiczii (Hook. f.) Wunderlin	Ncn	P	Altos de Campana Nat. Park (trail)	FLORPAN 5895	Wh	EtOH	<100	<100
Rytidostylis trianaei (Cogn.) Kuntze.	Ncn	C	Bogota Savannah	COL 498050	L	EtOH	>100	>100
Ericaceae								
Cavendishia bracteata (Ruiz & Pavón ex J. St.-Hil.) Hoerold	Ncn	C	Bogota Savannah	COL 498051	L	EtOH	>100	>100
Vaccinium poasanum Donn. Sm	Ncn	CR	Tapanti Nat. Park	INB LDV 0773	A	EtOH	>100	>100
Euphorbiaceae								
Acalypha macrostachya Jacq.	Ncn	CR	Carara Nat. Park	INB LDV 0780	Ro	EtOH	>100	>100
Adelia triloba (Müll. Arg.) Hemsl.	Clavillo	CR	Carara Nat. Park	INB LA 0804	Ro	EtOH	>100	>100
Croton guatemalensis Lotsy	Copalchi	G	Cerro Gordo, SR	CFEH 964	B	EtOH	>100	>100
Pedilanthus tithymaloides (L.) Poit.	Pie de niño	P	Chiriqui, Ngöbe	FLORPAN 6006	L	EtOH	<100	<100
Ephedraceae								
Ephedra tweediana Fisch. & C.A. Mey.	Tramontana	A	El Palmar, Entre Ríos	ERA 4940	L/F	EtOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL Ss	MIC µg/mL Fp
Fabaceae								
Acacia caven (Molina) Molina	Espinillo, aromo	A	San Luis, Capital	UNSL Del Vitto & Petenatti 6256	L	EtOH	>100	>100
Acosmium panamense (Benth.) Yakovlev	Ncn	CR	Lomas de Barbudal Biol. Reserve	INB LA 3471	Ro	EtOH	>100	>100
Astragalus pehuenches Niederl.	Hierba loca	A	Mendoza, Malargue, Las Cuevas	UNSL Del Vitto & Petenatti 8636	F	EtOH	>100	>100
					S	EtOH	<100	<100
Cassia grandis L. f.	Carao	G	Samayac, Such.	CFEH 379	L	EtOH	>100	>100
Diphysa robinioides Benth.	Guachipilín	G	Samayac, Such.	CFEH	L	EtOH	>100	>100
Hymenaea courbaril L.	Guapinol	G	Cuilco, Huehue	CFEH 309	L	EtOH	>100	>100
Senna alata (L.) Roxb.	Mamuri aqui	B	Abel Iturralde, La Paz	LPB GB 1777	L	EtOH	>100	>100
Senna occidentalis L.	Frijolillo	G	Samayac, Such.	CFEH 342	L	EtOH	>100	>100
Vicia faba L.	Haba	G	Guatemala City	CFEH 360	L	EtOH	>100	>100
Fagaceae								
Quercus crispifolia Trelease.	Encino	G	Sierra de las Minas	CFEH 604	L	EtOH	>100	>100
					S	EtOH	>100	>100
Quercus oleoides Schltdl. & Cham	Roble encimo	CR	Santa Rosa Nat. Park	LA 3461	W	EtOH	>100	>100
Humiriaceae								
Humiriastrum diguense (Cuatrec.) Cuatrec.	Chiricano	CR	Piedras Blancas Nat. Park	INB LA 1586	A	EtOH	>100	>100
Hydrophyllaceae								
Wigandia urens (Ruiz & Pav.) Kunth var.*caracasana* (Kunth) D.N. Gibson	Chocón	G	Cerro Alux, Mixco	CFEH 989	F	EtOH	>100	>100
Lamiaceae								
Salvia lavanduloides Kunth	Salvia	G	Tacaná, SM	CFEH 735	L/F	EtOH	>100	>100
Teucrium grisebachii (Epling) Hieron. ex Epling.	Ajenjo	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 5766	L	MeOH	>100	>100
Liliaceae								
Zuccagnia punctata Cav.	Jarilla macho, pus-pus.	A	San Luis, Belgrano, Ayacucho	UNSL Del Vitto & Petenatti 9230	L	MeOH	50	>100
Loranthaceae								
Struthanthus orbicularis (Kunth) Blume	Ncn	P	Interamerican road, by Esso gas station	FLORPAN 6538	Wh	EtOH	>100	>100
Tripodanthus flagellaris (Cham. & Schltdl.) Tiegh.	Liga blanca	A	San Luis, Capital, Juana Koslay	UNSL Del Vitto & Petenatti 9244	Wh	EtOH	100	>100
Lythraceae								
Cuphea glutinosa Cham. & Schltdl.	Siete sangrías	A	Junín, Merlo, San Luis	UNSL Del Vitto & Petenatti 8544	L	MeOH	>100	>100
Malvaceae								
Wercklea insignis Pittier & Standl.	Ncn	CR	Braulio Carrillo Nat. Park	INB LDV 0739	B	EtOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL Ss	MIC µg/mL Fp
Marantaceae								
Thalia geniculata L.	Ncn	CR	Palo Verde Nat. Park	INB LA 3468	Wh	EtOH	>100	>100
Melastomataceae								
Monochaetum myrtoideum (Bonpl.) Naudin	Saltón	C	Bogota Savannah	COL 498053	L/S	EtOH	100	>100
					L	CH_2Cl_2	>100	>100
Menispermaceae								
Cissampelos tropaeolifolia DC.	Alcotán	G	Livingston, Izabal	CFEH 451	L	EtOH	>100	>100
Moraceae								
Brosimum alicastrum Sw.	Ncn	B	Franz Tamayo, La Paz	LPB DQ 1550	B	EtOH	>100	>100
Pseudolmedia laevis (Ruiz & Pav.) J.F. Macbr	Nui	B	Abel Iturralde, La Paz	LPB SD 147	B	EtOH	>100	>100
Myrtacee								
Blepharocalyx gigantea Lillo	Anacahuita	A	Rosario City	UNR MG 815	L	MeOH	>100	>100
Myristicaceae								
Virola koschnyi Warb.	Ncn	CR	La Cangreja Nat. Park	INB LA 3476	L	EtOH	>100	>100
Onagraceae								
Fuchsia boliviana Carrière	Ncn	C	Bogota Savannah	COL 498054	L	EtOH	>100	>100
Orchidaceae								
Epidendrum mosenii Barb. Rodr.	Ncn	Br	Itajaí, SC	HBR VCFilho 003	F	MeOH	>100	50
					L	MeOH	100	>100
Papaveraceae								
Argemone subfusiformis G.B. Ownbey	Cardo santo	B	Aguarati/Ibasiriri,Cordillera, Santa Cruz	LPB GB 2053	F	MeOH	>100	>100
					L/F	MeOH	100	>100
Phytolaccaceae								
Phytolacca bogotensis Kunth	Guaba, cargamanta	A	Misiones, Gral. Belgrano	UNR MG 121	L	CH_2Cl_2	12.5	>100
Phytolacca tetramera Hauman	Ombusillo	A	La Plata-BA Road	UNR MG 134	S	MeOH	>100	>100
					Fr	MeOH	>100	>100
					Rh	MeOH	>100	>100
					L	MeOH	100	>100
Trichostigma octandrum (L.) H. Walter	Bejuco de palma	A	Iguazú Nat. Park	UNR SI 447	L	H_2O	>100	12.5
Seguieria aculeata Jacq.	Zarzahueca	A	Salta, Orán, El Rey #18, Isla de Cañas	UNR MG 119	L	MeOH	>100	>100
					L	CH_2Cl_2	<100	<100
Piperaceae								
Piper amalago L.	Cordoncillo	G	Lachuá, AV	CFEH 960	L	EtOH	100	>100
					S	EtOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL Ss	MIC µg/mL Fp
Piper barbatum Kunth.	Cordoncillo	C	Mosquera-La Mesa Km. 20	COL 499616	L	EA	>100	>100
						EtOH	>100	<100
Piper jacquemontianum Kunth.	Cordoncillo	G	Lachuá, AV	CFEH 959	L	EtOH	>100	>100
						Hexane	>100	>100
						CH_2Cl_2	>100	>100
						EA	>100	>100
Piper scabrum Lam.	Cordoncillo	G	Lachuá, AV	CFEH 961	L	EtOH	25	>100
Poaceae								
Gynerium sagittatum (Aubl.) P. Beauv.	Caña brava	CR	Rainy Forest Reserve	INB AR 5424	A	EtOH	>100	>100
Polygalaceae								
Monnina xalapensis Kunth	Cinco negritos	G	El Caracol, Quiche	CFEH 882	L	EtOH	50	100
					S	EtOH	12.5	100
Polygonaceae								
Polygonum acuminatum Kunth.	Catay grande	A	Puerto Gaboto, Sta. Fe	UNR SG 763	L	MeOH	100	>100
Polygonum punctatum Elliot	Catay dulce	A	San Luis, Pringle, Río Grande	UNSL Del Vitto & Petenatti 7848	L/F	MeOH	>100	>100
Polygonum ferrugineum Wedd.	Caatay guazú	A	Puerto Gaboto, Sta. Fe	UNR SG 757	L	MeOH	100	>100
Triplaris americana L.	Anani	B	Abel Iturralde, La Paz	LPB SD 124	B	EtOH	>100	>100
Polypodiaceae								
Phlebodium decumanum Willd.	Calahuala	H	Yohoa lake	CFEH 683	L	EtOH	>100	>100
					Rh	EtOH	>100	>100
Phlebodium pseudoaureum (Cav.) Lellinger .	Calahuala	G	Mataquescuintla, Jal	CFEH 1039	Rh	EtOH	>100	>100
					L	EtOH	>100	>100
Portulacaceae								
Portulaca oleracea L.	Verdolaga	P	Colón, Costa Arriba	FLORPAN 5926	Wp	EtOH	100	100
Rhamnaceae								
Karwinskia calderonii Standl.	Guiligüiste	CR	Santa Rosa Nat. Park	INB NZ 2119	W	EtOH	100	>100
Scutia buxifolia Reiss.	Coronilla	A	Entre Ríos, La Paz	ERA 38	L/F	MeOH	>100	>100
Ranuculaceae								
Clematis montevidensis Spreng.	Cabello de ángel	A	San Luis, Capital	UNSL Del Vitto & Petenatti 5091	F	MeOH	>100	>100
					L/F	MeOH	>100	>100
Rosaceae								
Crataegus pubescens (C. Presl.) C. Presl.	Manzanita	G	Chiquimula		Fr	EtOH	12.5	100
Rubiaceae								
Alibertia edulis (Rich.) A. Rich. Ex DC.	Trompito	P	Soberanía Nat. Park	FLORPAN 6547	S	EtOH	>100	>100
					L	EtOH	>100	>100
Faramea correae C.M. Taylor	Ncn	P	Altos de Campana Nat. Park (trail)	FLORPAN 6540	S	EtOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL Ss	MIC µg/mL Fp
					L	EtOH	>100	>100
Faramea eurycarpa Donn. Sm.	Ncn	P	Altos de Campana Nat. Park (La Cruz)	FLORPAN 6539	L	EtOH	>100	>100
					S	EtOH	>100	>100
Galium latoramosum Clos	Ncn	A	San Luis, San Martín, La Huertita	UNSL Del Vitto & Petenatti 5635	L/F	MeOH	>100	>100
Isertia laevis (Triana) B. M. Boom	Ncn	P	Cerro Azul.	FLORPAN 2177	L	EtOH	>100	>100
					S	EtOH	>100	>100
Ladenbergia bullata (Wedd.) Standl.	Ncn	P	Llano-Cartí, Km. 12.2	FLORPAN 5166	L	EtOH	>100	>100
					S	EtOH	>100	>100
Palicourea guianensis Aubl.	Ncn	P	Soberanía Nat. Park	FLORPAN 6549	L	EtOH	>100	>100
					F	EtOH	>100	>100
					S	EtOH	>100	>100
Psychotria psychotriifolia (Seem) Stand.	Ncn	P	Colón, Guanche river	FLORPAN 5923	L	EtOH	>100	>100
					S	EtOH	>100	>100
Quiinaceae								
Quina florida Tul.	Quina-quina	B	Abel Iturralde, La Paz	LPB SD 418	L	EtOH	>100	>100
Rutaceae								
Amyris pinnata Kunth	Ncn	CR	Guanacaste Nat. Park	INB LA 3459	L	EtOH	50	>100
Erythrochiton gymnanthus Kallunki	Cafecillo	CR	Carara Nat. Park	INB LA 0739	Ro	EtOH	>100	>100
Santalaceae								
Acanthosyris falcata Griseb	Madroño	A	San Cristóbal, Sta. Fe	UNR NC 7931	L	MeOH	>100	100
Sapindaceae								
Sapindus saponaria L.	Jaboncillo	B	Abel Iturralde, La Paz	LPB AS 197	Fr	MeOH	>100	>100
Schizaeaceae								
Lygodium venustum Sw.	Ncn	P	Soberanía Nat. Park	FLORPAN 5908	A	EtOH	<100	<100
Smilacaceae								
Smilax domingensis Willd.	Zarzaparrilla	G	Samayac, Such.	CFEH 662	L	EtOH	>100	>100
					S	EtOH	>100	>100
					Rh	EtOH	>100	>100
					F	EtOH	>100	>100
Solanaceae								
Cestrum nocturnum L.	Ncn	P	Colón, Guanche river	FLORPAN 5922	L	EtOH	<100	<100
Cestrum parqui L'Her.	Hediondilla, palqui	A	San Luis, Capital, Cruz de Piedra	UNSL Del Vitto & Petenatti 4383	F	MeOH	50	>100
					F dry	MeOH	>100	>100
					F fresh	MeOH	>100	>100
Lycianthes synanthera (Sendtn.) Bitter	Quelite	G	Samayac, Such.	CFEH 1067	L	EtOH	>100	>100
Lycium cuneatum Dammer	Ncn	A	Castellanos, Sta Fe	UNR 3818	L/F	MeOH	>100	>100

Table 1. continued on next page

Table 1. Continued.

Family/Specie	Name	Country	Place of collection	Voucher number	Part	Solvent	MIC µg/mL	
							Ss	Fp
Physalis viscosa L.	Camambú	A	San Luis, Junín, Merlo	UNSL Del Vitto & Petenatti 8579	Rh	MeOH	>100	>100
					L	MeOH	>100	>100
					F	MeOH	>100	>100
Solanum americanum Miller	Macuy	G	Samayac, Such.	CFEH 401	L	EtOH	>100	>100
Solanum cornifolium Dunal	Tinto	C	Bogota Savannah	COL 498583	L	EtOH	>100	>100
Sterculiaceae								
Sterculia apetala (Jacq.) H. Karst.	Castaño	G	Flores, Petén	CFEH 280	L	EtOH	>100	>100
					B	EtOH	>100	>100
Tiliaceae								
Heliocarpus appendiculatus Turcz.	Burío	CR	Braulio Carrillo Nat. Park	INB PH 0034	W	EtOH	>100	>100
Urticaceae								
Parietaria debilis G. Forst.	Rompepiedra	A	San Luis Capital	UNSL Del Vitto & Petenatti 7563	L/F	EtOH	>100	>100
Phenax mexicanus Wedd.	Agüilla	CR	Braulio Carrillo Nat. Park	INB AR 5709	A	EtOH	>100	>100
Valerianaceae								
Valeriana prionophylla Standl.	Valeriana	G	Nebaj, Quiché	CFEH 906	Ro	EtOH	100	>100
Verbenaceae								
Cornutia pyramidata L.	Lat che	G	Cobán, AV	CFEH 724	L	EtOH	>100	>100
Lippia graveolens Kunth	Orégano	G	Subinal, El Progreso	CFEH 456	L	EtOH	25	100
Verbena litoralis Kunth	Verbena	G	Guatemala City	CFEH 876	L	EtOH	>100	>100
Vitex megapotamica (Spreng.) Moldenke	Tarumán	A	Sto. Tomé, Arroyo	IBONE 4605	L	MeOH	>100	>100
Amphotericin B							6.25	12.5

*Ncn, No common name; because this sample was collected by random bioprospection in protected reserves.
Country = A, Argentina; Br., Brazil; B, Bolivia; C, Colombia; CR, Costa Rica; G, Guatemala; H, Honduras; P, Panama.
Parts used = A, Aerial parts; B, Bark; F, Flowers; Fr, Fruits; L, Leaves; Rh, Rhizome; Ro, Roots; S, Stalk; V, Vine; W, Wood; Wh, Whole plant.
Solvent = EA, Ethyl acetate; EtOH, Ethanol; MeOH, Methanol.
Fungi = Ss, *Sporothrix schenckii,* Fp, *Fonsecaea pedrosoi.*

(Vanbreuseghem et al., 1970) were inoculated with the fungi and incubated at 27°C for 21 days; the spores were removed with sterile saline solution, counted, and a suspension containing 100 spores/μL was prepared. For screening, tubes containing 13.5 mL of Sabouraud agar were autoclaved, cooled to 50°C, 1.5 mL of an extract containing 1000 μg/mL were added, poured into Petri dishes, cooled, and incubated at 35°C to check for sterility (final concentration 100 μg/mL). Four equidistant holes were made in each agar-extract dish with sterile Durham tubes; 30 μL of the spore suspension were placed in each hole, incubated al 27°C for 21 days for *S. schenckii* and 28 days for *F. pedrosoi*; as controls, dishes containing 35% ethanol (growth 100%) and 100 μg/mL amphotericin B (growth 0%) were prepared. Amphotericin B was chosen as positive control since it is used as drug for the treatment of both fungi (Kohler et al., 2007; López Martínez & Mendez Tovar, 2007). The colony diameter was measured in millimeter using a vernier and an extract was considered active when it showed 75% inhibition of the colony diameter in comparison with the control. For minimum inhibitory concentration (MIC), extract-agar dishes were challenged with fungal spores in the same manner as above, but using serial dilutions of extracts in the agar dishes (Gaitán et al., 2007).

Results

Different parts of the plants were collected either from biodiversity plots or based on ethnomedical use. One hundred ninety five extracts from 151 plant species were evaluated by the agar dilution method at a fixed screening concentration of 100 μg/mL. At this concentration, 28 extracts showed activity (MIC ≤100 μg/mL), 13 against both fungi, 11 against only *S. schenckii,* and 4 against *F. pedrosoi*. Then the MIC of each of the active extract was assessed with the agar dilution assay according to the method of Gaitán et al. (2007). Of these, four extracts from *Gnaphalium gaudichaudianum* DC (Asteraceae), *Plumeria rubra* L (Apocynaceae), *Tecoma stans* (L.) Juss. ex Kunth. (Bignoniaceae) and *Trichostigma octandum* (L.), H. Walter showed activity against *F. pedrosoi* at MIC 12.5 μg/mL, and four extracts from *Bourreria huanita* (Lex.) Hemsl. (Boraginaceae), *Phytolacca bogotensis* Kunth (Phytolaccaceae), *Monnina xalapensis* Kunth (Polygalaceae) and *Crataegus pubescens* (C. Presl) C. Presl (Rosaceae) showed activity against *S. schenckii*; and the activity of 10 extracts could not be confirmed due to insufficient material (Table 1). No significant difference was found among plants chosen on the basis of ethnomedical use (41/112, 36.6%) or collected from biodiversity plots (13/39, 33.3%) (Z test of two independent populations, $P = 0.823$).

For interpretation of results, we considered that an extract was inactive when MIC was >100 μg/mL, moderately active when MIC was 25–50 μg/mL and strongly active when MIC was 12.5 μg/mL. Although several extracts (167, 85.6%) did not show any activity against the studied fungi, the eight species mentioned above showed strong activity and others showed moderate activity, such as *Terminalia triflora* (Griseb.) Lillo (Combretaceae), which showed an MIC of 25 μg/mL against both fungi, and *Aristolochia gibertii* Hook. (Aristolochiaceae), *Piper scabrum* Lam (Piperaceae), and *Lippia graveolens* Kunth. (Verbenaceae) were active against *S. schenckii* at MIC of 25 μg/mL.

Discussion

Only 18 (9.2%) extracts showed inhibitory activity against one or both of the subcutaneous fungi, *S. schenkii* and *F. pedrosoi* studied (MIC ≤50 μg/mL). Eight (4.1%) extracts were active at MIC of 12.5 μg/mL. Only one extract of the flower of *B. huanita* inhibited both fungi. This is a rare tree native to Mesoamerica, and its flowers are used popularly for skin ailments and ulcers (Orellana, 1987), as well as for several nervous ailments (Torres, 2007). Previous information on this plant is negligible; one study showed lack of antifungal activity in the ethanol extract of the flowers against *Candida, Aspergillus* and dermatophytes (Svetaz et al., 2010).

G. gaudichaudianum is a herb native to Argentina, where it is used in folk medicine for the treatment of respiratory diseases and as a vulnerary, but no activity has been demonstrated previously against a panel of pathogenic fungi (Svetaz et al., 2010).

T. triflora is a tree native to South America used in folk medicine as antiseptic, but results of its antifungal activity are controversial, since in one study the methanol extract showed activity against *Trichophyton mentagrophytes, T. rubrum* (100 μg/mL) and *Microsporum gypseum* (250 μg/mL) (Muschietti et al., 2005), while another study showed no antifungal activity (Svetaz et al., 2010). Other important activities have been demonstrated by the aqueous extract of leaves, such as pro-oxidant activity (Desmarchelier et al., 1997), and inhibition of polymerase and ribonuclease activities of HIV reverse transcriptase at IC_{50} 1.6–1.8 μg/mL (Martino et al., 2002); by bioguided fractionation, active principles were shown to be punicalin and 2-*O*-galloylpunicalin (Martino et al., 2004). Other *Terminalia* spp. have shown activity against *S. schenckii* at 20–40 μg/mL such as extracts of seven South African species (Masoko et al., 2005), as well as the acetone extract of *T. sambesiaca* Engl. & Diels, and hexane and acetone extracts of *T. phanerophlebia* Engl. & Diels (30 μg/mL) (Shai et al., 2008); from stem bark of *T. barachystemma* was isolated punicalagin with important activity against *Candida* spp. (Liu et al., 2009).

The other five species showed activity, but only to one of the fungi. In the case of *P. bogotensis* no specific information was found, but phytolaccosides isolated from *Phytolacca tetramera* Hauman showed activity against a panel of opportunistic fungi (Escalante et al., 2002); from *P. rubra*, plumericin and isoplumericin were isolated, showing molluscicidal and antibiotic activity (Hamburger et al., 1991); and, the chloroform extract of *T. stans* gave

the best inhibition zone against fungal activity (Gandhi Ramesh, 2010), but no activity was shown against pathogenic fungi (Svetaz et al., 2010). From *C. pubescens, M. xalapensis,* and *T. octandrum*, no reference of biocidal activity was detected in the literature.

We describe here for the first time the inhibitory activity of extracts from *G. gaudichaudianum*, *P. rubra*, *T. stans* and *T. octandrum* against *F. pedrosoi*, and from *B. huanita, C. pubescen*, *M. xalapensis* and *P. bogotensis*, against *S. schenckii*.

Conclusion

It is noteworthy that three species showed good activity, not described before, against two subcutaneous fungi, *S. schenkii* and *F. pedrosoi,* which are usually resistant to most of the available antibiotics, *B. huanita* (MIC: 12.5 and 25 μg/mL, respectively), *G. gaudichaudianum* (MIC: 50 and 12.5 μg/mL, respectively), and *T. triflora* (MIC 25 μg/mL to both fungi). The antifungal activity of extracts from *P. rubra*, *T. stans* and *T. octandrum* against *F. pedrosoi* and from *C. pubescen, M. xalapensis* and *P. bogotensis*, against *S. schenckii*, is reported for the first time. Only in 36% of plants screened the found activity in this study may explain their ethnomedical use.

Declaration of interest

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