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RESEARCH ARTICLE

Screening of Latin American plants for antiparasitic activities against malaria, Chagas disease, and leishmaniasis

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Abstract

In order to explore rationally the medical potential of the plant biodiversity of the Central and South American region as a source of novel antiparasitic molecules, a multinational Organization of American States (OAS) project, which included the participation of multidisciplinary research centers from Argentina, Bolivia, Colombia, Costa Rica, Guatemala, Nicaragua and Panama, was carried out during the period 2001-2004. This project aimed at screening organic plant extracts for antitrypanosomal, antileishmanial and antimalarial activities and subsequently isolating and characterizing bioactive molecules. Plants for antiparasitic screening were selected from a database of ethnomedical uses of Latin American plants (PlanMedia) based on the amount of biological and chemical information available in the literature. We report here the evaluation of 452 extracts from 311 plant species *in vitro* screens against *Plasmodium falciparum, Leishmania mexicana,* and *Trypanosoma cruzi*. Out of 311 species tested, 17 plants (5.4%) showed antiparasitic activities at IC_{50} values ≤ 10 µg/mL. The most active plants were *Acnistus arborescens* (L.) Schltdl. (Solanaceae) (leaf, EtOH, IC_{50}: 4 µg/mL) *Monochaetum myrtoideum* Naudin (Melastomataceae) (leaf, MeOH, IC_{50}: 5 µg/mL) and *Bourreria huanita* (Lex.) Hemsl. (Boraginaceae) (branch, EtOH, IC_{50}: 6 µg/mL). These were selectively active against *P. falciparum, L. mexicana* and *T. cruzi*, respectively.

Keywords: *Malaria; Chagas disease; leishmaniasis; Latin America; screening; antiparasitic activity*

Introduction

Malaria, trypanosomiasis, and leishmaniasis have burdened the Latin American and Caribbean regions for centuries and have negatively influenced their ability to develop and become competitive societies in the current climate of globalization (Hotez et al., 2008). American trypanosomiasis (also known as Chagas disease) is caused by *Trypanosoma cruzi* and is the major endemic disease in Latin America.

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Malaria is a major global public health problem responsible for approximately one million cases per year in the Americas. The increasing global spread of drug resistance to most of the available and affordable antimalarial drugs is a major concern and requires innovative strategies for combating the disease. Leishmaniasis is a vector-borne disease, affecting 72 developing countries and in three Latin American countries. Visceral leishmaniasis caused by *Leishmania donovani* is the most severe form of *Leishmania* infections.

The current chemotherapy regimens for these parasitic diseases are limited and are not ideal because of the often associated severe side effects. The emergence of drug-resistant parasites presents an additional and major problem. All these facts underline the urgent need for the development of new, cheap, safe and easy-to-administer molecules for the treatment of these tropical diseases. Considering the great potential of Latin America in terms of its untapped plant biodiversity and rich traditional medicine, this study was undertaken to screen selected plants for *in vitro* antiplasmodial, antileishmanial, and antitrypanosomal activities and subsequently isolate the bioactive molecules.

Materials

Plant material

Plants were collected mainly from tropical forests throughout the seven countries (Argentina, Bolivia, Colombia, Costa Rica, Guatemala, Nicaragua and Panama). Their taxonomic identity was established by the botanists Martha Gattuso, M. Frecentese, Elisa Petenatti (Argentina); Rosy de Michel, Genevieve Bourdy, Andrés Roca (Bolivia); Ricardo Callejas, Edgar Linares, José Luis Fernández, Zaleth Cordero, Santiago Díaz, N.R. Salinas (Colombia), Luis Guillermo Acosta, Alexander Rodríguez (Costa Rica); Mario Veliz, Elfriede de Pöll, Juan José Castillo (Guatemala); Ricardo Rueda, Dania Paguaga, Hilario Mendoza, Nelson Toval y Miguel Garmendia (Nicaragua); Mireya Correa (Panamá). Voucher specimens are deposited in the corresponding National Herbarium in each country: Herbarium of the National University of San Luis (UNSL), Argentina; Herbarium of the Universidad Nacional de Rosario (UNR), Argentina; National Herbarium of Bolivia (HLP), Bolivia; National Colombian Herbarium (COL), Colombia; Herbarium of INBio(INB), Costa Rica; CEMAT-FARMAYA Ethnobotany Herbarium, (CFEH) Guatemala; Universidad Nacional Autónoma de Nicaragua-León (Herbario UNAN-León), (HULE), Nicaragua; and Herbarium of the Universidad de Panamá (PMA), Panama.

Selection of plants

A random list of plants from the ethnomedical database PlanMedia (CIFLORPAN, 2004) of public information was submitted to a NAPRALERT (Natural Products Alert database, University of Illinois at Chicago) search for biological and chemical information. From that list, 311 plant species were prioritized according to the reported ethnomedical uses for three neglected diseases and lack of chemical and biological information.

Preparation of extracts

In general, plant material was macerated with 80% ethanol, methanol, dichloromethane, chloroform and hexane (24 h) for extraction (3 times). The plant extracts were filtered and concentrated *in vacuo* at < 40°C in a rotary evaporator and stored at -80°C until tested.

Biological assays

Antiplasmodial assay

Antiplasmodial activity was determined in a chloroquine-resistant *P. falciparum* strain (W2 Indochina) utilizing a novel microfluorimetric assay to measure the inhibition of the parasite growth based on the detection of the parasitic DNA by intercalation using Pico Green (Corbett et al., 2004). The IC_{50} values were calculated from relative fluorescence units as compared with untreated controls. The parasites were maintained at 2% hematocrit in flat-bottom flasks (75 mL) in human red blood cells from O positive blood type donors with RPMI 1640 medium (Gibco BRL) supplemented with 10% O positive human serum. Chloroquine was used as a standard antimalarial drug and showed an IC_{50} value of 65-100 nM.

Antileishmanial assay

The study used a fluorimetric *Leishmania mexicana* (WHO-MOHM/B2/82/BELZ) axenic amastigote assay adapted from a novel antimalarial micro-method by Corbett et al. (2004), to measure inhibition of the parasite growth based on the detection of parasitic DNA by intercalation with PicoGreen. Briefly, 25,000 amastigotes from axenic culture (Bates, 1993) were co-incubated with the test substances at 32°C in a CO_2-free atmosphere for 72 h. The relative fluorescence units (RFU) were quantified with a fluorescence microplate reader (FLx800; Bio-Tek Instruments, Winooski, VT) at 485/20 nm excitation and 528/20 nm emission filters. Amphotericin B was used as a standard anti-leishmanial agent and had an IC_{50} value of 100 nM.

Antitrypanosomal assay

The recombinant Tulahuen clone C4 of *Trypanosoma cruzi,* which expresses β-galactosidase (βGal) as a reporter enzyme was used in the assay (Buckner et al., 1996). The method was based on the growth inhibition effect of the test samples on trypomastigote, the bloodstream form of the parasite. The resulting color from the cleavage of chlorophenol red-β-galactopyranoside (CPRG) by βGal, expressed by the parasite, was measured at 570 nm as indirect measurement of parasite growth. The inhibitory concentration of 50% growth (IC_{50}) as compared with the untreated control was calculated from the optical density values. Assays were conducted at 37°C under an atmosphere of 5% CO_2/95% air mixture. Nifurtimox was tested as a standard antitrypanosomal agent and had an IC_{50} value in the range of 10 nM.

Results

Antiparasitic activities of extracts

Four hundred fifty-two extracts were prepared from 311 plants and were first tested at the concentration of 50 μg/mL on a chloroquine-resistant *P. falciparum* strain (W2 Indochina), *Leishmania mexicana* (WHO-MOHM/B2/82/BELZ), and *Trypanosoma cruzi*. Of plants tested at this stage 49% belong to the major families such as Asteraceae, Piperaceae, Rubiaceae, Solanaceae, and Fabaceae. Ninety-four extracts representing 86 plants, 68 genera and 39 families showed $IC_{50} \leq 50$ μg/mL (Table 1). The *in vitro* antitrypanosomal, antiplasmodial and antileishmanial activities of the extracts were qualified as most active when IC_{50} values were ≤ 10 μg/mL.

Antitrypanosomal activity

Nine plant extracts showed $IC_{50} \leq 10$ μg/mL. The most active plants against *T. cruzi* were *Acnistus arborescens* (leaf, EtOH), *Scoparia dulcis* L. (Scrophulariaceae) (whole plant, EtOH) and *Maianthemum paludicola* La Frankie (Convallariaceae) (whole plant, MeOH), with IC_{50} values of 4, 4 and 5 μg/ml, respectively. *Chromolaena leivensis* (Hieron.) R.M. King & H. Rob (Asteraceae) (aerial parts, EtOH) showed antitrypanosomal activity at IC_{50} value of 8 μg/mL, whereas *Annona muricata* L. (Annonaceae) (leaf, EtOH), *Argemone subfusiformis* G.B. Ownbey (Papaveraceae) (fruit, MeOH) and *Caesalpinia paraguariensis* (D. Parodi) Burkart (Fabaceae) (leaf, EtOH), *Piper barbatum* Kunth (Piperaceae) (leaf, EtOH) and *Piper holtonii* C. DC. (Piperaceae) (root, EtOH) displayed the same activity at IC_{50} value of 10 μg/mL. The antitrypanosomal activity of these species has not been reported previously in the literature except in the case of *Annona muricata,* which has been used in the folk medicine of Nicaragua for diarrhea (Coee & Anderson, 1996) and for fever in Colombia (Blair et al., 1991). Its antitrypanosomal activity against epimastigotes was reported previously (Abe et al., 2005). The 95% ethanol extract of seeds of *Annona cherimola* Mill. (Annonaceae) displayed antitrypanosomal activity against *T. cruzi* (Kim et al., 2007). Stem bark (petroleum ether extract) of *Annona senegalensis* Pers. (Annonaceae) showed activity against *Trypanosoma brucei* at MIC of 19 μg/mL (Freiburghaus et al., 1996).

The Izoceño-guarani use *Argemone subfusiformis* against cough, cold, and flu in Bolivia (Bourdy, 2002), and intestinal worms in Argentina (Martínez Crovetto, 1981).

Chromolaena leivensis has been used traditionally as febrifuge (Moreno & Tinjaca, 1986). A related species *C. christieana* (Baker) R.M. King & H. Rob. (Asteraceae) (stem and bark) showed the highest percentage of lysis on bloodstream forms of *T. cruzi* at a concentration of 250 μg/mL (Rojas de Arias et al., 1995).

Piper holtonii and *P. barbatum* have been used in Colombian traditional medicine for fever and malaria (García-Barriga, 1975). Five chromenes were isolated from *Piper gaudichaudianum* Kunth and *Piper aduncum* L. (Piperaceae) and displayed *in vitro* activity against epimastigote forms of *T. cruzi* (Batista et al., 2008). Neolignans isolated from leaves of *Piper regnellii* (Miq.) C. DC. var. *pallescens* (C. DC.) Yunck (Piperaceae) showed *in vitro* antiproliferative effects against *T. cruzi* (Luize et al., 2005, 2006). Although *Piper glabratum* Kunth and *P. acutifolium* Ruiz & Pav. (Piperaceae) were not active in our screening, two benzoic acid derivatives with moderate activity against *P. falciparum* were isolated and reported by Flores et al. (2008).

Scoparia dulcis (leaves), called "tupeicha" in Bolivia (Licorice weed or Sweet broom), has been used in traditional medicine of the Guarani for dysentery, diarrhea, stomachache and fever (Bourdy, 2002).

Caesalpinia paraguariensis, commonly known as "ivirayepiro", has been used traditionally by the Guarani ethnic group in Paraguay to treat dysentery, bloody diarrhea, furuncles, fever, stomach ache and body ache (Bourdy, 2002). Norcaesalpinin E, a furanocassane-type diterpene, isolated from *Caesalpinia crista* L. (Fabaceae) (seed kernels) showed a higher antimalarial activity (IC_{50} value = 0.090 μM) than chloroquine (Linn et al., 2005; Kalauni et al., 2006). Five out of nine active antitrypanosomal plants from this study were previously reported for their ethnomedical uses to treat symptoms related to Chagas disease.

Table 1. Active antiparasitic plants.

Family/Species	Vernacular name	Voucher no.;Date and place of collection	Part	Solvent	IC_{50} (μg/mL)		
					T. cruzi	*P. falciparum*	*Leishmania*
Anacardiaceae							
Lithrea molleoides (Vell.) Engl.	*Molle de beber, molle dulce*	5339 (UNSL); 10/03/2002, Suyuque Nuevo, Dpto. Belgrano, San Luis, Argentina	AP	MeOH	>50	32	>50
Schinus molle L.	*Aguaribay, gualeguay, terebinto*	6154 (UNR); 18/11/2003, Santa Fe, Argentina	AP	MeOH	>50	25	>50
Annonaceae							
Annona muricata L.	*Guanábana*	COL 504946; 31/07/2002; Vereda Andalucía, Anapoima/Cundinamarca, Colombia	LF	EtOH	10	20	>50
Sapranthus viridiflorus G. E. Schatz		LA 0775; 04/2000; Parque Nacional Carara, Costa Rica	AP	MeOH	25	13	36
Apocynaceae							
Rauvolfia tetraphylla L.	*Chalchupa*	437; 2002, Samayac, Mazatenango, Guatemala	RT	EtOH	>50	17	40
Aquifoliaceae							
Ilex guayusa Loes.	*Guayusa*	HPUJ 011734; 15/02/2001; Putumayo, Colombia	LF	EtOH	47	>50	>50
Arecaceae							
Euterpe precatoria Mart.	*Palmito*	LA 3456; 07/2004; Reserva Biológica Bosque Lluvioso, Costa Rica	R	MeOH	>50	16	>50
Asclepiadaceae							
Sarcostemma gracile Decne.	*Sapua Kati*	GB2147; 05/2001, Prov. Cordillera, Bolivia	AP	EtOH	42	>50	23
Asteraceae							
Baccharis notosergila Griseb.	*Tipisha*	7931 (UNR); 18/11/2003, Arminda Dto. Rosario Pcia. Santa Fe, Argentina	AP	MeOH	>50	45	>50
Baccharis trinervis Pers.	*Santo Domingo*	643; 2001, Alta Verapaz, Guatemala	LF	EtOH	>50	>50	29
Calea peruviana (Kunth) Benth. ex S.F. Blake	*Carrasposa*	27/07/2001; Sabana de Bogotá/ Cundinamarca, Colombia	LF	EtOH	>50	22	>50
Calea jamaicensis (L.) L.	*Carrasposa*	COL 468655; 27/07/2001; Cunday/ Cundinamarca, Colombia	AP	EtOH	30	>50	>50
Chromolaena leivensis (Hieron.) R.M. King & H. Rob.	*Sanalo-todo*	COL 504952;31/07/2002; Vía Mosquera-La Mesa/Cundinamarca, Colombia	AP	EtOH	8	>50	>50
Critonia morifolia (Mill.) R.M. King & H. Rob.	*Lengua de vaca*	COL 504880;11/03/2003; Km. 73, Carretera Mosquera - La Mesa, Cundinamarca, Colombia	FR	EtOH	29	17	>50
Eirmocephala brachiata (Benth.) H. Rob.		COL 498588; 03/03/2004; Cundinamarca, Colombia	LF	EtOH	33	31	>50
Egletes viscosa (L.) Less.	*Kaami*	GB 2077; 05/2001, Prov. Cordillera, Bolivia	WP	EtOH	38	>50	>50
Gnaphalium gaudichaudianum DC.	*Marcela, marcela macho, marcelita*	9239 (UNSL); 10/10/2002, Juana Koslay, Dpto Capital, San Luis, Argentina	AP	MeOH	36	>50	>50
Gochnatia glutinosa (D. Don) ex Hook. & Arn.	*Jarilla, jarillilla, jarilla sacancia*	8909 (UNSL); 20/05/2002, La Calera, Dpto. Belgrano, San Luis, Argentina	AP	MeOH	20	>50	38
Mikania periplocifolia Hook. & Arn.	*Guaco*	8521(UNSL); 10/03/2000, Valle de Pancanta, Dpto. Pringles, San Luis, Argentina	AP	MeOH	>50	20	7
Solidago chilensis Meyen var. *megapotamica* (DC.) Cabrera	*Vara de Oro*	MG 257 (UNR); 11/2003, Pérez, Santa Fe, Argentina	LF	MeOH	32	>50	>50
			FW	MeOH	37	>50	>50

Table 1. Continued on next page.

Table 1. Continued.

Family/Species	Vernacular name	Voucher no.;Date and place of collection	Part	Solvent	IC_{50} (μg/mL)		
					T. cruzi	*P. falciparum*	*Leishmania*
Tagetes filifolia Lag.	*Anisillo*	9243 (UNSL); 8/05/2003, Río Grande - Dpto. Pringles - San Luis, Argentina	AP	MeOH	>50	39	18
Bignoniaceae							
Jacaranda mimosifolia D. Don	*Jacarandá, tarco, caroa*	9371 (UNSL); 31/03/2003, La Capital, San Luis, Argentina	LF	MeOH	>50	38	>50
Tynanthus guatemalensis Donn. Sm.	*Bejuco de agua*	925; 2004, Petén, Guatemala	ST	EtOH	>50	32	>50
Boraginaceae							
Bourreria huanita (Lex.) Hemsl.	*Esquisuchil*	895; 2003, Antigua Guatemala, Sacatepéquez, Guatemala	LF	EtOH	>50	>50	12
			BR	EtOH	>50	>50	6
Bourreria spathulata (Miers) Hemsl.	*Campa-cambao*	9237 (UNSL); 19/03/2003, Juana Koslay, Dpto Capital, San Luis, Argentina	LF	MeOH	30	8	36
Cordia cylindrostachya (Ruiz & Pav.) Roem. & Schult.	*Leño negro, hierba de golondrina*	COL 498048; 03/03/2004; San Francisco, Cundinamarca	LF	EtOH	35	26	>50
Bromeliaceae							
Aechmea distichantha Lem.	*Caraguatá*	2303 (UNR); 01/11/2003, Las Gamas, Dto. Vera, Pcia. Santa Fe, Argentina	LF	MeOH	48	>50	>50
Capparaceae							
Capparis salicifolia Griseb.	*Ivaguasu*	GB-2034; 05/2001, Prov. Cordillera, Bolivia	LF	EtOH	39	>50	21
Combretaceae							
Combretum laxum Jacq.		35249 (IBONE); 10/6/2001, Misiones, Argentina	AP	MeOH	34	>50	>50
Terminalia triflora (Griseb.) Lillo		35248; 28/02/2003, Las Gamas, Dto. Vera, Pcia. Santa Fe, Argentina	AP	MeOH	>50	9	41
Convallariaceae							
Maianthemum paludicola La Frankie		LA 3270; 12/2003; Reserva Forestal Los Santos, Costa Rica	WP	MeOH	5	>50	>50
Convolvulaceae							
Ipomoea carnea subsp. *fistulosa* (Mart. ex Choisy) D.F. Austin	*Tararaki*	GB 2057; 05/2001, Prov. Cordillera, Bolivia	LF	EtOH	48	18	>50
Euphorbiaceae							
Sebastiania brasiliensis Spreng.	*Mata ura, ibirá camby, lecherón, palo leche*	1652 (E.R.A.); 02/2003, Paraná, Argentina	AP	MeOH	>50	15	>50
Sebastiania commersoniana (Baill.) L.B. Sm. & Downs		1634 E.R.A.; 27/01/2003, Paraná, Argentina	AP	MeOH	>50	26	>50
Fabaceae							
Astragalus pehuenches Niederl.	*Yerba loca, tembladerilla*	8637 (UNSL); 17/04/2002, Las Cuevas, Dpto Malargüe, Mendoza, Argentina	BK	MeOH	>50	48	>50
Caesalpinia paraguariensis (D. Parodi) Burkart	*Ivirayepiro*	RM-2571; 05/2001, Prov. Cordillera, Bolivia	LF	EtOH	10	>50	>50
Piscidia carthagenensis Jacq.	*Cachimbo, pellejo de toro*	LA 3256; 11/2003; Parque Nacional Palo Verde, Costa Rica	AP	MeOH	28	37	29
Zuccagnia punctata Cav.	*Jarilla macho, jarilla de la puna, laca, pus-pus*	8912 (UNSL); 07/01/2004, San Luis, Argentina	LF	EtOH	20	35	>50
Flacourtiaceae							
Lozania pittieri (S.F. Blake) L.B. Sm.		LDV 0798; 10/2004; Parque Nacional Braulio Carrillo, Costa Rica	LF	MeOH	30	>50	>50

Table 1. Continued on next page.

Table 1. Continued.

Family/Species	Vernacular name	Voucher no.;Date and place of collection	Part	Solvent	IC_{50} (μg/mL)		
					T. cruzi	*P. falciparum*	*Leishmania*
Loranthaceae							
Psittacanthus cordatus (Hoffmanns. ex Schult. f.) Blume	*Nderirembui*	RM-2700; 09/2000, Bolivia	LF	EtOH	40	>50	>50
Melastomataceae							
Miconia buxifolia Naudin	*Gallinazo*	COL 471732; 06/11/2001; Vía Mosquera-La Mesa/Cundinamarca, Colombia	LF	EtOH	>50	24	>50
Monochaetum myrtoideum (Bonpl.) Naudin	*Saltón*	COL 498053; 03/05/2004; Bojacá, Vereda San Antonio, Cundinamarca, Colombia	LF	EtOH	>50	5	>50
Menispermaceae							
Cissampelos tropaeolifolia DC.	*Alcotan*	541; 2004, Livingston, Izabal, Guatemala	LF	EtOH	>50	25	>50
Moraceae							
Clarisia biflora Ruiz & Pav.	*Lechillo, ojoche negro*	LA 3284; 12/2003; Parque Nacional Carara, Costa Rica	AP	MeOH	25	>50	>50
Myrsinaceae							
Ardisia densiflora Krug & Urb.	*Chipe*	2001, Sierra de las Minas, Chiquimula, Guatemala	LF	EtOH	>50	>50	23
			BK	EtOH	>50	19	37
Myrtaceae							
Blepharocalyx salicifolius (Kunth) O. Berg	*Horco molle, Anacahuita*	MG 815 (UNR); 10/11/2003, Tucumán, Argentina	LF	MeOH	39	>50	>50
Myrcianthes rhopaloides (Kunth) McVaugh	*Guayabillo*	LA 2908; 11/2000; Parque Nacional Tapantí, Costa Rica	AP	MeOH	24	>50	>50
Onagraceae							
Fuchsia boliviana Carrière	*Fucsia*	COL 498054; 03/05/2004; Vereda de San Antonio, Bojacá, Cundinamarca, Colombia	LF	EtOH	>50	20	>50
Hauya lucida Donn. Sm. & Rose	*Subús, tubús*	LA 2948; 11/2000; Parque Nacional La Cangreja, Costa Rica	AP	MeOH	32	17	36
Papaveraceae							
Argemone subfusiformis G.B. Ownbey	*Cardo santo, cardo amarillo*	7463 (UNSL); 10/11/2002, La Calera, Dto. Belgrano, Prov. San Luis, Argentina	FR	MeOH	10	>50	>50
			FW	MeOH	21	>50	>50
Bocconia integrifolia Bonpl.		11/03/2003; Km. 10 carretera Mosquera - La Mesa, Cundinamarca, Colombia	LF	EtOH	>50	23	>50
Phytolaccaceae							
Phytolacca bogotensis Kunth	*Ombusillo*	MG 121 (UNR); 15/06/2003, Gral Belgrano, Misiones, Argentina	AP	MeOH	>50	>50	31
Phytolacca tetramera Hauman	*Ombusillo*	MG 134 (UNR); 20/02/2003, La Plata, Buenos Aires, Argentina	AP	MeOH	>50	>50	20
Piperaceae							
Piper acutifolium Ruiz & Pav.	*Thuda*	GB1640; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	39	19	>50
Piper aduncum L.	*Thuda*	GB1893 06/2001 Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	38	21	>50
Piper aeruginosibaccum Trel.	*Cordoncillo*	959; 2002, Lachuá, Alta Verapaz, Guatemala	LF	EtOH	12	11	24
			LF	Hexane	24	14	>50
			LF	EtoAc	22	22	>50
			LF	$CHCl_3$	8	11	>50
Piper barbatum Kunth	*Cordoncillo*	COL 499616; 04/02/2002; El Triunfo, Cundinamarca, Colombia	LF	EtOH	10	6	>50
Piper dilatatum Rich.	*Matico*	GB1100; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	31	12	>50

Table 1. Continued on next page.

Table 1. Continued.

Family/Species	Vernacular name	Voucher no.;Date and place of collection	Part	Solvent	IC_{50} (μg/mL)		
					T. cruzi	*P. falciparum*	*Leishmania*
Piper elongatum Vahl	*Matico*	GB1644; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	36	17	>50
Piper glabratum Kunth	*Thuda*	GB 1877; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	25	14	>50
Piper hispidum Sw.	*Matico*	GB1300; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	26	22	>50
Piper holtonii C. DC.	*Cordoncillo*	COL 468663; 01/06/2001; El Triunfo/ Cundinamarca	RT	EtOH	10	12	>50
Piper longestylosum C. DC.	*Thuda*	GB1500; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	>50	14	>50
Piper pilliraneum C. DC	*Thuda*	GB1716; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	35	17	>50
Piper rusbyi C. DC.	*Thuda*	GB1614; 07/2002, Prov. A. Iturralde, Bolivia	LF	CH_2Cl_2	32	25	>50
Piper scabrum Lam.	*Cordoncillo*	961; 2003, Lachuá, Alta Verapaz, Guatemala	LF	EtOH	32	26	>50
Piper umbellatum L.	*Cordoncillo*	04/02/2002; Anapoima/Cundinamarca, Colombia	LF	EtOH	25	>50	>50
Polygonaceae							
Polygonum acuminatum Kunth	*Sanguinaria del agua*	SG 763 (UNR); 10/03/2003, Puerto Gaboto - Santa Fe, Argentina	LF	MeOH	>50	8	>50
Polygonum ferrugineum Wedd.	*Caatay guazu*	SG 757 (UNR); 25/11/2002, Puerto Gaboto - Santa Fe, Argentina	AP	MeOH	37	>50	>50
Ranunculaceae							
Clematis campestris A. St.-Hil.	*Cabello de angel*	5061(UNSL); 15/09/1999, Rodeo del Alto, Dpto. La Capital, San Luis, Argentina	FW (fresh)	MeOH	>50	9	>50
			AP	MeOH	30	19	>50
Rhamnaceae							
Ziziphus mistol Griseb.	*Yua*	RM2570; 05/2001, Prov. Cordillera, Bolivia	LF	EtOH	25	25	>50
Rosaceae							
Crataegus pubescens (C. Presl) C. Presl	*Manzanita*	2003, Quetzaltepeque, Chiquimula, Guatemala	FR	EtOH	>50	>50	30
Rubiaceae							
Cinchona pubescens Vahl	*Quina*	11/03/2003; Km. 74 Carretera Mosquera - La Mesa, Cundinamarca, Colombia	LF		>50	27	>50
Galium latoramosum Clos		Muñoz 356 (E.R.A.); 15/09/2002, Concordia- Entre Ríos- Argentina	AP	MeOH	>50	>50	14
Scrophulariaceae							
Capraria biflora L.	*Tupeicha*	RM 2554; 05/2001, Prov. Cordillera, Bolivia	AP	EtOH	46	>50	>50
Scoparia dulcis L.	*Tupeicha*	RM 2528; 05/2001, Prov. Cordillera, Bolivia	WP	EtOH	4	>50	>50
Solanaceae							
Acnistus arborescens (L.) Schltdl.	*Tomatoquina*	COL 359328; 12/01/2001; San Francisco, Cundinamarca, Colombia	LF	EtOH	4	>50	>50
Cestrum parqui L'Hér.	*Palque, hediondilla, duraznillo negro*	8780 (UNSL); 10/05/2002, El Volcán, Dpto. La Capital, San Luis, Argentina	AP	MeOH	>50	>50	28
			FW (fresh)	MeOH	>50	>50	24
Lycium cuneatum Dammer		3818 (UNR); 10/11/2003, Venados Grandes, Chaco, Argentina	AP	MeOH	29	>50	>50
Nicotiana glauca Graham	*Palán, palán-palán*	9372 (UNSL); 20/03/2005, La Capital, San Luis, Argentina	AP	MeOH	38	24	>50
Solanum cornifolium Dunal	*Tinto*	COL 498583; 03/03/2004; Km 20, Carretera Bogotá Choachí, Cundinamarca, Colombia	LF	EtOH	>50	12	>50
Styracaceae							
Styrax conterminus Donn. Sm.	*Copalillo*	2001, Sierra de las Minas, Chiquimula, Guatemala	BK	EtOH	>50	>50	14
Urticaceae							

Table 1. Continued on next page.

Table 1. Continued.

Family/Species	Vernacular name	Voucher no./Date and place of collection	Part	Solvent	IC_{50} (μg/mL) *T. cruzi*	*P. falciparum*	*Leishmania*
Parietaria debilis G. Forst.	*Parietaria, paletaria, anisillo*	3882 (UNSL); 10/03/2001, San Roque, La Capital, San Luis, Argentina	AP	MeOH	>50	>50	8
Verbenaceae							
Lippia graveolens Kunth	*Orégano mexicano*	456; 2003, Samayac, Mazatenango, Guatemala	LF	EtOH	13	>50	>50
Phyla nodiflora (L.) Greene		LA 3469; 09/04; Parque Nacional Palo Verde, Costa Rica	WP	MeOH	30	13	>50
Zygophyllaceae							
Larrea cuneifolia Cav.	*Jarilla macho*	6437 (UNSL); 07/01/2004, Sierras de la Quijada, San Luis, Argentina	AP	MeOH	40	12	>50
Chloroquine					0.045		
Amphotericin B						0.086	
Nifurtimox							4.0

Plant part: AP, aerial parts; BK, bark; FR, fruit; LF, leaf; RT, root; ST, stems; FW, flowers; WP, whole plant; BR, branch.
Solvent: ethanol, EtOH; methanol, MeOH; ethyl acetate, EtoAc; chloroform, $CHCl_3$; dichloromethane, CH_2Cl_2.

Antiplasmodial activity

Five plant extracts showed activity at $IC_{50} \leq 10$ μg/mL. The most active plants were *Monochaetum myrtoideum* (leaf, EtOH), *Bourreria spathulata* (Miers) Hemsl. (Boraginaceae), (leaf, EtOH), *Polygonum acuminatum* Kunth (Polygonaceae) (leaf, MeOH), *Clematis campestris* A. St.-Hil. (Ranunculaceae) (flower, MeOH) and *Terminalia triflora* (Griseb.) Lillo (Combretaceae) (aerial parts, MeOH) at IC_{50} values of 5, 8, 8, 9 and 9 μg/ml, respectively. There is no report on the chemical composition of *Terminalia triflora*, but other species such as *Terminalia glaucescens* Planch. ex Benth. (Combretaceae) used in West African traditional medicine for malaria has shown *in vitro* antiplasmodial activity (IC_{50} 2.34-4.83 μg/ml) against various *Plasmodium falciparum* strains (Mustofa et al. 2000). An aqueous extract of *Terminalia macroptera* Guill. & Perr. (Combretaceae) (used in traditional medicine in Burkina Faso) displayed activity against *Plasmodium falciparum* chloroquine-resistant W2 strain ($IC_{50} = 1$ μg/ml) (Sanon et al., 2003). Polar extracts of *T. glaucescens* (leaf and stem) showed antiplasmodial activity against chloroquine-resistant *P. falciparum* at IC_{50} values between 0.4-8 μg/ml (Mustofa et al., 2000).

Species of *Polygonum*, *Polygonum senegalense* Meisn. (Polygonaceae), displayed antiplasmodial activity due to the presence of 9-hydroxyhomoisoflavanone in aerial exudates (Midiwo et al., 2007). Polar extracts of *Polygonum multiflorum* Thunb. (Polygonaceae) root showed antiplasmodial activity against chloroquine-resistant *P. falciparum* (Tran et al., 2003). Species of *Monochaetum* from Colombia have been used in traditional medicine for fever and paludism (García-Barriga, 1975).

This is the first report of the antimalarial activity of *Monochaetum myrtoideum*, *Bourreria spathulata*, *Polygonum acuminatum*, and *Terminalia triflora*. The bioactive compounds responsible for this activity have not yet been reported. The ethnomedical uses of only one out of five antiplasmodial plants correlate with their observed biological activity.

Antileishmanial activity

Three plant extracts showed activity at IC_{50} value of ≤ 10 μg/mL. The most active plants were *Bourreria huanita* (bark, EtOH), *Mikania periplocifolia* Hook. & Arn. (Asteraceae) (aerial parts, MeOH) and *Parietaria debilis* G. Forst. (Urticaceae) (aerial parts, MeOH) at IC_{50} values of 6, 7 and 8 μg/ml, respectively. This is the first report of the antileishmanial activity of these three plants; no compound has been identified as responsible for this biological activity so far.

Mikania periplocifolia is used as febrifuge in Argentina (Zardini, 1984). A related species, *M. congesta* DC. (Asteraceae), has also been reported with the same ethnobotanical use in Peru (Roumy et al., 2007). Another species, *Mikania, M. glomerata* Spreng. (Asteraceae) showed significant effects against axenic amastigote and promastigote forms of *Leishmania* (L.) *amazonensis* at a concentration of 100 μg/mL with a percentage of growth inhibition between 49.5 and 99% (Luize et al., 2005). *Parietaria debilis* is used traditionally for washing blisters in Argentina (Martínez Crovetto, 1981). The ethnomedical uses of two out of three antileishmanial plants are in agreement with the results of this study.

Conclusions

This study has identified a number of plant extracts (Table 1) that have shown *in vitro* antiparasitic activities,

based on the selection of plants listed in the database PLANMEDIA. The results of the antiparasitic screening revealed that 2 out of 17 active plants belong to the genus *Piper*. In the species examined, their ethnomedical uses show a good correlation with the observed antiparasitic activity. These are under study to isolate active compounds.

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Declaration of interest

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