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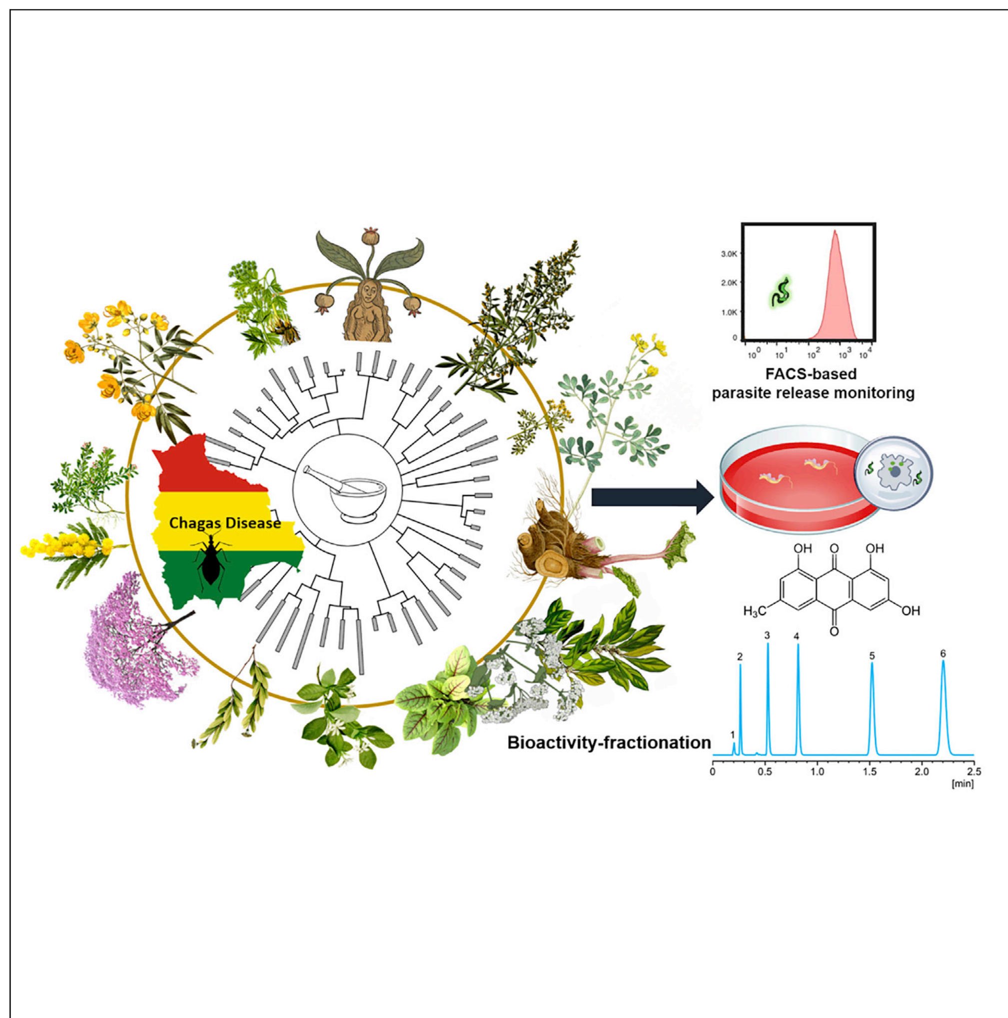
Phylobioactive hotspots in plant resources used to treat Chagas disease



Andrea Salm, Sandhya R. Krishnan, Marta Collu, ..., Marco Leonti, Giovanna Almanza, Jürg Gertsch

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Highlights

Ethnopharmacological fieldwork on antichagasic medicinal plants in Bolivia

Bioprospecting of natural product classes effective against *Trypanosoma cruzi*

Isolation of natural products able to inhibit *T. cruzi* host cell infection

Structure-activity relationship study on the antichagasic effects of anthraquinones

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Phylobioactive hotspots in plant resources used to treat Chagas disease

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SUMMARY

Globally, more than six million people are infected with *Trypanosoma cruzi*, the causative protozoan parasite of the vector-borne Chagas disease (CD). We conducted a cross-sectional ethnopharmacological field study in Bolivia among different ethnic groups where CD is hyperendemic. A total of 775 extracts of botanical drugs used in Bolivia in the context of CD and botanical drugs from unrelated indications from the Mediterranean *De Materia Medica* compiled by Dioscorides two thousand years ago were profiled in a multidimensional assay uncovering different antichagasic natural product classes. Intriguingly, the phylobioactive anthraquinone hotspot matched the antichagasic activity of *Senna chloroclada*, the taxon with the strongest ethnomedical consensus for treating CD among the Izoceño-Guaraní. Testing common 9,10-anthracenedione derivatives in *T. cruzi* cellular infection assays demarcates hydroxyanthraquinone as a potential antichagasic lead scaffold. Our study systematically uncovers *in vitro* antichagasic phylogenetic hotspots in the plant kingdom as a potential resource for drug discovery based on ethnopharmacological hypotheses.

INTRODUCTION

Chagas disease (CD) or American trypanosomiasis is the disease caused by infection of the hemoflagellate protozoan *Trypanosoma cruzi* (Pérez-Molina and Molina, 2018). The parasitic *T. cruzi* is mainly transmitted to humans by hematophagous reduviid bugs of the subfamily Triatominae (*Triatoma* spp.) (Barrett et al., 2003; Coura and Borges-Pereira, 2010; Pérez-Molina and Molina, 2018). Other means of transmission involve blood transfusion, organ transplant, congenital, and oral contamination (Coura and Borges-Pereira, 2010; Pérez-Molina and Molina, 2018). CD is classified as one of the most neglected tropical diseases, especially among low-income populations (Conteh et al., 2010). The World Health Organization estimates that about 6–7 million people are infected worldwide, primarily in Latin America, where the disease is endemic (WHO, 2018). Bolivia has the highest CD incidence in the world (Organización Panamericana de la Salud, 2006; WHO, 2015), and *T. cruzi* infection has a high prevalence in different rural areas, primarily affecting the indigenous communities in the inter-Andean valleys and the Chaco (Cassab et al., 1999; Organización Panamericana de la Salud, 2006; de Araújo-Jorge and Medrano-Mercado, 2009).

CD presents two clinical phases. The acute stage of the disease (i.e., infection) often remains unperceived and only very rarely morbidity and mortality occur (Teixeira et al., 2006). In some cases, the sites of bug bites get inflamed and form nodules called chagoma or "Romaña sign" when the protozoan enters via the conjunctiva. After an incubation period of less than two weeks, newly infected individuals may develop fever, chills, myalgia, rash, or meningeal irritation. However, the initial chagoma may be the only symptom of *T. cruzi* infection for years. At the chronic stage, parasitism and inflammation of the heart and/or enlarged colon can result in severe pathophysiological endpoints, including megacolon, megaesophagus, and cardiomyopathy (Rassi et al., 2010). During the chronic phase, *T. cruzi* infection can develop an indeterminate form, where, depending on the *T. cruzi* strain and possibly host immunity, approximately 70% of the infected individuals remain completely asymptomatic (Barrett et al., 2003; Coura and Borges-Pereira, 2010; Ribeiro et al., 2012). Currently, two approved drugs are used to treat acute CD, namely the nitroheterocyclic compounds nifurtimox (Lampit) and benznidazole (Rochagan). However, there is a controversy about their efficacy in chronic CD(Coura and de Castro, 2002; Marin-Neto et al., 2009), and both drugs show adverse effects upon prolonged administration, such as abdominal pain, headache, and neutropenia

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(Castro et al., 2006; Jackson et al., 2010). Moreover, these antichagasic drugs are hardly accessible for the indigenous communities in Bolivia. Consequently, people living in rural areas of Bolivia rely on traditional medicines for their primary health care (Quiroga et al., 2012; Vandebroek et al., 2008). Botanical drugs contribute significantly to Bolivian folk medicine and they are harvested wild, cultivated, and traded at local markets (Bussmann et al., 2016). Despite reports making general claims about the efficacy of traditional botanical drugs (Cordell and Colvard, 2005; Gertsch, 2009), few studies address their mode of action based on comparative hypothesis-driven ethnopharmacological research. It is unknown whether CD, which has a millennial history in the area (Aufderheide et al., 2004), enforced a selection pressure to prompt ethnomedical strategies among the affected indigenous groups in Bolivia capable of reducing parasitemia or primarily treat the symptoms of CD.

Plants are known to produce a high diversity of secondary metabolites showing numerous pharmacological effects, including antiparasitic and antimicrobial (Wink, 2012; Newman and Cragg, 2020). Medicinal plants may act via different mechanisms of action also against various causative agents of neglected tropical diseases like *Mycobacterium ulcerans* or *T. cruzi* (Llurba-Montesino et al., 2015; Zimmermann et al., 2013; Tsouh Fokou et al., 2015). Plant secondary metabolites have led to the development of antiprotozoal chemotherapeutics (Cheuka et al., 2016). Ethnopharmacology continues to inspire bioprospecting as it investigates treatment consensus of natural drugs among ethnic groups as anecdotal indications for presumed pharmacological efficacy (Buenz et al., 2018; Trotter and Logan, 1986). Considering Bolivia's rich biocultural diversity and the endemic nature of CD, ethnopharmacological and bioprospecting studies focusing on antichagasic remedies are surprisingly scarce(Calderón et al., 2010; de Arias et al., 1994; Fournet et al., 1994; Muñoz Ortiz et al., 2010). In parallel, few studies were dedicated to the ethnomedicine and management of CD in Bolivia (Bastien, 1998; Forsyth, 2017). Although numerous plant extracts and natural products have been reported to exert moderate (IC_{50} values $\leq$100 μg/mL) to significant (IC_{50} values 15 $\leq$ μg/mL) selective toxicity toward different strains of *T. cruzi* epimastigotes (Izumi et al., 2011; Muschietti and Ulloa, 2016; Schmidt et al., 2012a, 2012b), reports on activity against trypomastigote cellular infection and amastigote replication *in vitro* are relatively rare. Overall, systematic and comparative antitrypanosomal screenings with comprehensive extract libraries derived from botanical drugs are lacking, thus hindering a direct comparison of efficacy within the same assay.

Here, we report a cross-sectional ethnopharmacological field study among the indigenous ethnic groups Quechua, Izoceño-Guaraní, Ayoreo, and Chiquitano in Bolivia with the aim to investigate their prevalent ethnomedical strategies to treat CD. We document the knowledge about botanical drugs used for the treatment of CD-related symptoms. Fieldwork involved structured and semi-structured interviews, as well as plant collections (drug samples and herbarium vouchers), which was followed by taxonomical identification. An ethyl acetate (EtOAc) extract library of the botanical drugs used to treat CD was generated for *in vitro* testing against trypanosomes. To validate the ethno-directed bioprospecting approach, we also profiled *in vitro* antitrypanosomal effects of EtOAc extracts obtained from Mediterranean botanical drugs described in *De Materia Medica* (*DMM*), written by Dioscorides in the first century AD (Staub et al., 2016). We hypothesized that the CD botanical drug library would result in a comparatively higher number of extracts showing selective antichagasic activity *in vitro*. We assembled bioactive (i.e. antichagasic) plant extracts into phylogenetic groups reflecting chemotaxonomic relationships, a process we designated "phylobioactivity". Employing phylobioactivity-guided grouping in combination with high pressure liquid chromatography (HPLC)-based activity profiling of active extracts, we tested whether bioactive phylogenetic clusters could serve as a basis for more efficient characterizations of antichagasic plant metabolites. The screening of the *DMM* extract library showed phylogenetic patterns of activity reflecting chemical associations. This multidimensional profiling resulted in a preliminary structure-activity relationship (SAR) study on the antichagasic effects of anthraquinones on parasite release upon host cell infection by *T. cruzi* trypomastigotes *in vitro*.

RESULTS

Ethnopharmacological survey in Bolivia

Ethnomedical data were obtained from 361 informants in three different geographic areas representing four ethnic groups (Figure 1). In total, 152 research participants (5 Ayoreo, 68 Chiquitano, 54 Izoceño-Guaraní, 19 Quechua, and six sellers from the city markets) reported ethnomedical knowledge related to the treatment of CD. In our study, the gender of the informants was uniformly distributed, and we were not able to detect gender-specific knowledge related to the treatment of CD (Table 1). When asked about

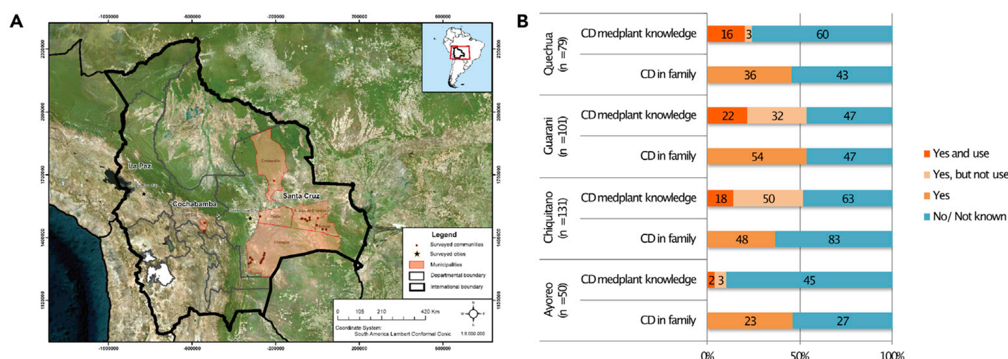

Figure 1. Ethnopharmacological survey in Bolivia

(A) The three different municipalities (orange), surveyed communities (red dots), and cities (stars) where the field study was conducted are shown.

(B) Graphical summary of the ethnomedical data. The number of informants from the four ethnic groups (Ayoreo, Chiquitano, Guaraní, and Quechua) reporting knowledge of medicinal plants/agents for CD treatment (CD medplant knowledge) and the reported occurrence of CD in the family (CD in family) are shown. See also Figure S1.

CD and its symptoms, informants of all ethnic groups showed a lack of ethnomedical knowledge related to the pathophysiology of CD. Unlike other disease categories, such as dermatological affections or diseases of the digestive system, chronic CD was recognized unambiguously only after diagnosis by blood tests in the health centers or hospitals. Symptoms related to cardiomyopathies (general fatigue, shortness of breath, dizziness, chest pain, heart palpitations, high blood pressure, and swelling of feet and legs) were generally associated with CD among the Chiquitano and Izoceño-Guaraní who enriched their ethnomedical knowledge by information obtained from medical doctors. Botanical drugs used for CD were most frequently associated with these symptoms.

The survey resulted in more than 350 use reports for 79 plant taxa used in the context of CD. Of these, 69 were identified to the species level, nine plants were identified to the genus level, and one taxon was identified only to the family level. The recorded remedies comprised plant taxa distributed across 37 botanical families and 74 genera (Table 2). From the plants identified to the species level, 80% were native to Bolivia. Only 41 medicinal plant species (52%) were collected from the wild, while the others were obtained through cultivation or from local markets. Decoctions and infusions in cold and hot water, respectively, were the most common preparation forms for oral administration. Informants reported that the chronic symptoms were relieved after taking these plant remedies.

The most cited plant species in the treatment of CD-related symptoms were *Senna chloroclada* (flowers, root) (Figure S1) and *Tabebuia aurea* (bark), followed by the introduced and globally known medicinal plant species *Alpinia zerumbet* (rhizome) and *Cymbopogon citratus* (herb). Noteworthy, Chiquitano informants also reported a blend of several botanical drugs against CD, such as a combination of the barks of *Jacaranda cuspidifolia*, *T. aurea*, and *Trichilia* sp. Particularly interesting was that few informants stated that

Table 1. Sociodemographic characteristics of the informants in the surveyed rural areas reporting knowledge about medicinal plants for CD[a], number of use reports, and reported taxa

		Total	Gender		Age class			Use	Reported
Ethnicity	Geographical zone	informants	F	M	<40	41 to 60	>60	reports	taxa
Ayoreo	Chiquitania	5	3	2	1	4	0	5	3
Chiquitano	Chiquitania	68	33	35	22	24	22	199	38
Guaraní	Chaco	54	33	21	19	24	11	93	15
Quechua	Inter-Andean valleys	19	10	9	8	9	2	42	31
Total		146	79	67	50	61	35	339	87

[a]Responses of women sellers in herbal markets in La Paz and Santa Cruz cities are not included in this table.

Table 2. List of plant species reported to be used in the treatment of Chagas disease and related symptoms by Ayoreo, Chiquitano, Guaraní, and Quechua informants

Family genus/species	Vernacular name	Used by	Part used	No. use reports	Application	Origin (status)	Habit	Acute or chronic phase	Indication Chagas symptom	Voucher specimen
Amaranthaceae										
Dysphania ambrosioides (L.) Mosyakin & Clemants	Caré, paico	C	L, R	17	Or	N (Cul)	Herb	chro	CAR, GAS	ASMP33
Anacardiaceae										
Astronium urundeuva (Allemao) Engl.	Cuchi	C	B, L	2	Or	N (W)	Tree	chro	CAR	ASMP56, ASMP6
Schinus molle L.	Molle	Q	L	2	Or, Tp	N (W)	Tree	chro	CAR, GAS	ASMP90
Annonaceae										
Annona nutans (R. E. Fr.) R. E. Fr.	Sinini, Aratiku, Sorimimi	G	L	5	Or	N (W)	Shrub	chro	CAR	ASMP11
Duguetia sp.	Sinini	C	L	12	Or	n.d. (Cul)	Tree	chro	CAR	ASMP41
Apocynaceae										
Aspidosperma quebracho-blanco Schitdl.	Cacha	C	B	8	Or	N (W)	Tree	chro	CAR, FAT	ASMP35
Vallesia glabra (Cav.) Link	Amarguillo, Arakuarembiu	G	L	3	Or	N (W)	Tree	chro	CAR	ASMP17
Aristolochiaceae										
Aristolochia andina F. Gonzáles & I. Vargas	Waje	Q	Br, L	1	Or	N (W)	Vine	chro	CAR	ASMP94
Asteraceae										
Acanthostyles buniifolius (Hook. ex Hook. & Arn.) R.M. King & H. Rob.	Romero	Q	AP	2	Or	N (Cul)	Shrub	chro	FAT	ASMP82
Achyrocline alata (Kunth) DC.	Guacanqui, Wira wira	C, LP	AP	6	Or	N (Cul,Pur)	Herb	chro	CAR	ASMP45, ASMP103
Achyrocline hyperchlora S. F. Blake	Wira wira	Q	AP	2	Or	N (W)	Herb	chro	CAR	ASMP91
Ambrosia arborescens Mill.	Altamisa	Q	L	2	Or	N (W)	Subshrub	chro	CAR	ASMP83
Baccharis genistelloides (Lam.) Pers.	Carqueja, Kinsa k'ucho	LP, Q	AP	3	Or	N (Pur,W)	Herb	chro	CAR, GAS	ASMP28, ASMP85
Bidens andicola Kunth	Misuka	Q	AP	1	Or	N (W)	Herb	chro	PUR	ASMP89
Chromolaena connivens (Rusby) R.M. King & H. Rob.	Sunchuj maman	Q	Fr, L	1	Or	N (W)	Shrub	chro	FAT	ASMP75
Culcitium canescens Humb. & Bonpl.	K'ia k'ia	LP	AP	2	Or	N (Pur)	Herb	chro	CAR	ASMP102
Cynara sp.	Alcachofa	LP, SC	AP	2	Or	n.d. (Pur)	Herb	chro	CAR	ASMP13
Gochnatia boliviana S. F. Blake	Melinco	Q	L	1	Or	N (W)	Shrub	chro	CAR, FAT	ASMP87
Mutisia acuminata Ruiz & Pav.	Chinchirkuma	Q	Br, L	1	Or	N (W)	Shrub	chro	CAR	ASMP88
Parthenium hysterophorus L.	Artemisa, Chupurujumo	C	AP	1	Or	N (W)	Herb	acu, chro	FEV, PUR	ASMP59
Pluchea sagittalis (Lam.) Cabrera	4 Cantos	C	Fl, L	1	Or	N (Cul)	Shrub	chro	CAR	ASMP36

(*Continued on next page*)

Table 2. *Continued*

Family genus/species	Vernacular name	Used by	Part used	No. use reports	Application	Origin (status)	Habit	Acute or chronic phase	Indication Chagas symptom	Voucher specimen
Schkuhria pinnata (Lam.) Kunza ex Thell.	Jayaj pichana	Q	AP	2	Or	N (W)	Herb	chro	CAR, FAT	ASMP76
Sonchus oleraceus L.	Diente de Leon	C, LP	AP	3	Or	I (Pur)	Herb	chro	CAR	AMP12, ASMP30
Verbesina sp.	Tabaco	Q	L	1	Tp	n.d. (W)	Tree	acu	BIT	ASMP93
Bignoniaceae										
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Tajibo, Tajibo negro	C, G	B, Fl	3	Or	N (W)	Tree	chro	CAR	ASMP18, ASMP57
Jacaranda cuspidifolia Mart.	Paraparau	C	B	6	Or	N (W)	Tree	chro	CAR, FAT	ASMP34
Tabebuia aurea (Silva Manso) Benth. & Hook. F. ex S. Moore	Paratodo, Alcornoque	C, A	B	30	Or	N (Cul)	Tree	chro	CAR, FAT, PUR	ASMP9
Bixaceae										
Bixa orellana L.	Uruku	C, G	L	4	Or	N (W)	Tree	chro	CAR	ASMP26
Caricaceae										
Carica papaya L.	Papaya	C, G	Fl	3	Or	I (Cul)	Tree	chro	CAR, GAS	ASMP38
Cochlospermaceae										
Cochlospermum tetraporum Hallier f.	Kuari, Pela pela	G	B, L	8	Or	N (Cul)	Tree	chro	CAR	ASMP22
Cucurbitaceae										
Momordica charantia L.	Balsamina	C	AP	3	Or	I (Cul)	Vine	chro	CAR	ASMP58
Ephedraceae										
Ephedra americana Humb. & Bonpl. Ex Willd.	Pisqo simi	Q	AP	1	Or	N (W)	Shrub	chro	FAT	ASMP96
Euphorbiaceae										
Croton andinus Muell. Arg.	Taporita, Tupeicha	G	R	5	Or	N (W)	Herb	chro	CAR	ASMP21
Croton sp.	K'uru k'uru	Q	La	1	Tp	n.d. (W)	Herb	acu	BIT	ASMP92
Euphorbia serpens Kunth	Quebra Pedra	C	Wh	4	Or	N (Cul)	Herb	chro	CAR, FAT	ASMP49
Jatropha curcas L.	Piñón	C	L	1	Or	I (Cul)	Treelet	chro	FAT	ASMP39
Fabaceae										
Acacia aroma Gillies ex Hook. & Arn.	Tusca	G	B, Fl	2	Or	N (Cul)	Tree	chro	CAR	ASMP24
Acacia sp.	Karikari	C	B	1	Or	n.d. (Cul)	Tree	chro	PUR	ASMP43
Bauhinia sp.	Patecabra	C	L	1	Or	n.d. (Cul)	Shrub	acu, chro	FEV, PUR	ASMP42
Copaifera langsdorffii Desf.	Copaibo	C, SC	B	5	Or	N (W)	Tree	chro	CAR	ASMP51
Crotalaria incana L.	Amorocita	Q	L	1	Or	N (W)	Herb	chro	FAT	ASMP95
Hymenaea courbaril L.	Paquío	C	B	3	Or	N (W)	Tree	chro	CAR, FAT	ASMP55
Pterodon sp.	Pezoe	C	Se	2	Or	N (W)	Tree	chro	FAT	ASMP52

(*Continued on next page*)

Table 2. *Continued*

Family genus/species	Vernacular name	Used by	Part used	No. use reports	Application	Origin (status)	Habit	Acute or chronic phase	Indication Chagas symptom	Voucher specimen
Senna chloroclada (Harms) H. S. Irwin & Barneby	Lanza lanza, Mbuijare, Retama	G	Fl, R, Wh	45	Or	N (W)	Shrub	acu, chro	CAR, FEV	ASMP10
Spartium junceum L.	Retama	A, SC	AP	3	Or	I (Pur)	Shrub	chro	CAR	ASMP100
Gesneriaceae										
Gloxinia gymnostoma Griseb.	Ortelón	C	AP	2	Or	N (Cul)	Herb	chro	CAR, FAT	ASMP40
Gramineae										
Cymbopogon citratus (DC.) Stapf	Paja de cedrón	C, G, SC	L	25	Or	I (Cul)	Herb	chro	CAR, FAT	ASMP31
Labiatae										
Clinopodium axillare (Rusby) Harley	Huayra Muña	Q	Br, Fl, L	2	Or	N (Cul)	Subshrub	chro	CAR	ASMP80
Lepechinia vesiculosa (Benth.) Epling	Raq'acho	Q	L	1	Or	N (W)	Shrub	chro	CAR	ASMP81
Minthostachys ovata (Briq.) Epl.	Muña	Q	AP	3	Or	N (Cul)	Subshrub	chro	FAT, GAS	ASMP69
Ocimum americanum L.	Albahaca	C	Wh	2	Or	I (Cul)	Herb	chro	CAR	ASMP48
Aloe vera (L.) Burm.f.	Sábila, karaguata guasu	C, G	La	5	Tp	I (Cul)	Herb	acu	BIT, PUR	ASMP25
Linaceae										
Linum usitatissimum L.	Linaza	Q	Fr	2	Or	I (Cul)	Herb	chro	PUR	ASMP71
Malpighiaceae										
Galphimia brasiliensis (L.) A. Juss.	Masiaré	A, C	R	11	Or	N (Cul)	Herb	chro	CAR, FAT	ASMP32
sterile	Azucaró	C	B	4	Or	n.d. (W)	Tree	chro	CAR	ASMP54
Malvaceae										
Malva parviflora L.	Malva	LP, Q	AP	2	Or	I (Cul,Pur)	Herb	chro	CAR, FAT, GAS	ASMP77, ASMP15
Meliaceae										
Trichilia sp.	Tipa	C, SC	B	11	Or	n.d. (Cul)	Tree	chro	CAR	ASMP53
Myrtaceae										
Myrcianthes callicoma McVaugh	Guapurú	Q	L	1	Tp	N (W)	Tree	chro	FAT	ASMP73
Myrcianthes pseudomato (D. Legrand) McVaugh	K'arasacha	Q	L	2	Or	N (W)	Tree	chro	CAR, GAS	ASMP72
Plinia cauliflora (Mart.) Kausel	Guapurú	C	L	1	Or	N (Cul)	Tree	chro	CAR	ASMP60
Oxalidaceae										
Hypseocharis pimpinellifolia Remy	Sultaki	Q	R	1	Tp	N (W)	Herb	chro	FAT	ASMP70
Papaveraceae										
Argemone subfusiformis G. B. Ownbey	Cardo Santo	C, G	Fl	3		N (W)	Herb	chro	CAR	ASMP20
Bocconia integrifolia Bonpl.	Turumi	Q	L	1	Or	N (W)	Tree	chro	FAT	ASMP97

(*Continued on next page*)

Table 2. *Continued*

Family genus/species	Vernacular name	Used by	Part used	No. use reports	Application	Origin (status)	Habit	Acute or chronic phase	Indication Chagas symptom	Voucher specimen
Passifloraceae										
Passiflora cincinnata Mast.	Pachío, Mburukuya	C, G	Fl, L, R	13	Or	N (W)	Vine	chro	CAR, FAT	ASMP16
Piperaceae										
Piper rusbyi C. DC.	Matico	C	L	7	Or	N (Cul,W)	Shrub	chro	FAT, PUR	ASMP50, ASMP46
Plantaginaceae										
Plantago major L.	Llantén	C	Fl, L	3	Or	I (Cul)	Herb	chro	CAR	ASMP47
Polygalaceae										
Monnina wrightii A. Gray	T'ian t'ian	Q	L	1	Or	N (W)	Herb	chro	CAR	ASMP84
Rosaceae										
Rubus sp.	Kari kari	LP	AP	2	Or	n.d. (Pur)	Herb	chro	CAR	ASMP101
Rutaceae										
Heterophyllaea lycioides (Rusby) Sandwith	Kapi	Q	L	1	Or	N (W)	Shrub	chro	GAS	ASMP86
Ruta chalepensis L.	Ruda	Q	L	1	Or	I (Cul)	Herb	chro	GAS, PUR	ASMP99
Zanthoxylum coco Gillies ex Hook. F. & Arn.	Chirimolle	Q	L	1	Or	N (W)	Tree	chro	CAR	ASMP74
Solanaceae										
Cestrum parqui L'Her.	Andrés Huaylla	LP, Q	AP, L	2	Or	N (Cul,Pur)	Shrub	chro	CAR, GAS	ASMP78, ASMP14
Solanum palinacanthum Dunal	Pica Pica	C	L, R	1	Or	N (Cul)	Subshrub	chro	PUR	ASMP44
Urticaceae										
Urtica urens L.	Ortiga	LP	AP	1	Or	I (Pur)	Herb	chro	CAR	ASMP29
Valerianaceae										
Valeriana potopensis Briq.	Jama jama	Q	B, L	1	Or	N (W)	Herb	chro	CAR	ASMP98
Verbenaceae										
Aloysia citriodora Palau	Cedrón	C, Q	L	4	Or	N (Cul)	Shrub	chro	CAR	ASMP79
Aloysia polystachya (Griseb.) Moldenke	Poleo	C	AP	2	Or	N (Cul)	Shrub	chro	CAR	ASMP37
Zingiberaceae										
Alpinia zerumbet (Pers.) B. L. Burtt & R. M. Sm.	Colonia	C, G, SC	Fl	26	Or	I (Cul)	Herb	chro	CAR	ASMP19
Zygophyllaceae										
Bulnesia bonariensis Griseb	Guayacán, Guayacán Morado	C, G	B	3	Or	N (Cul)	Tree	chro	CAR	ASMP23

A, Ayoreo; C, Chiquitano; G, Guaraní; LP, La Paz; SC, Santa Cruz; AP, aerial parts; B, bark; Br, branches; Fl, flowers; Fr, fruit; La, latex; L, leaves; R, roots; Se, seeds; Wh, whole plant; Or, oral application; Tp, topical application; N, native; I, introduced; n.d., not determined; Cul, cultivated; Pur, purchased at market; W, wild; chro, chronic; acu, acute; BIT, vinchuca bite; CAR, cardiovascular symptoms; FEV, fever; GAS, gastro digestive symptoms; FAT, fatigue; PUR, purifier/blood purifier/to strengthen the blood.

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certain botanical drugs could cure chronic CD, namely *S. chloroclada* (flowers, root), *Cochlospermum tetraporum* (bark, leaves), and *Bulnesia bonariensis* (bark). However, there was no consensus among independent informants. Even during prolonged stays, the use of these remedies was rarely observed by the authors. Only the use of *Galphimia brasiliensis* (root) (Figure S1) could be repeatedly observed among the Chiquitano. To treat triatomine bites, topical applications in the form of poultices were mentioned (Table 2). Informants agreed that acute symptoms and inflammatory swellings (chagoma) at the bite sites occurred very rarely. Nocturnal triatomine bites were considered common and harmless. In highly affected communities (Izoceño-Guaraní and Ayoreo), informants stated that the bites were irritating but not painful and therefore remained mostly untreated. Only the taxa *Aloe vera*, *Croton* sp., and *Verbesina* sp were reported to be used for triatomine bites. *Bauhinia* sp., *S. chloroclada*, and *Parthenium hysterophorus* were mentioned as a treatment of fever during first infections. Besides plant-based remedies, eleven Chiquitano informants reported the use of animal products, such as *Coragyps atratus* (black vulture; blood), *Tapirus* sp. (tapir; nails), and *Equus africanus asinus* (donkey; milk) to treat the chronic symptoms of CD. Few Izoceño-Guaraní informants also reported the use of grease of armadillos (*Dasypoda* spp.) against triatomine bites, and others applied alcohol topically. The plant taxa used for CD obtained in markets were common medicinal plants known to be used for a variety of diseases and ailments (Table 2).

The Quechua and Ayoreo reported relatively fewer remedies to manage CD as compared to the Izoceño-Guaraní and Chiquitano (Table 1), despite the high prevalence of CD in their communities (>40%; Figure 1). The vast majority of the Ayoreo interviewed in this study did not report any botanical drugs used in the context of CD. The visited Ayoreo communities were evangelic or catholic Christians that stopped practicing shamanism decades ago. Generally, they had no or little knowledge about traditional medicinal agents. Although Quechua informants have been educated on the association between triatomine bugs and CD by institutional anti-CD campaigns (Salm and Gertsch, 2019), they had a very limited understanding of the transmission mechanisms and symptoms of the illness. There was poor consensus among Quechua informants, and we obtained only one or two use reports for each plant taxon, except for *Minthostachys ovata* aerial parts (3 use reports, Table 2). Most of the plants used by the Quechua were native to the region (86% of the total number of identified taxa at species level) and were gathered from the wild (74%). Among the Quechua, traditional healers (curanderos) generally carried out the preparation of botanical drugs. Although there were specialized healers among the Izoceño-Guaraní (paye) and Chiquitano communities, the knowledge and use of botanical drugs were not restricted to them. Guaraní and Chiquitano community members used botanical drugs to alleviate CD-associated symptoms because they are readily accessible and due to lack of alternatives. Especially among the Guaraní communities, there was a general lack of primary health care and traveling to urban centers for seeking medical care, posing significant difficulties for patients.

Identification of selective antitrypanosomal extracts from plant libraries

Biological profiling of the CD ethnobotanical drug library from Bolivia

Crude EtOAc extracts are obtained from 115 botanical drugs, representing 79 plant species and collected based on their use in the management of CD (Table 2). Although the ethnomedical extraction generally proceeded with hot water infusions, we justify the use of EtOAc for extract preparation by the reduced extraction of polar and high molecular weight compounds, such as sugars and tannins, which would potentially interfere with the screening. The extracts were dissolved in dimethyl sulfoxide (DMSO) and tested *in vitro* for their antitrypanosomal activity against *T. cruzi* epimastigotes (Y strain) and procyclic *T. b. brucei*, as well as for general cytotoxic effects in HeLa and Raw264.7 cells. Antiproliferative IC_{50} values <25 μg/mL were considered biologically significant. To define selective hits, a cutoff of 50% inhibition (HeLa antiproliferative effect) at 25 μg/mL was used. IC_{50} values were determined only for selective hits. Table S1 summarizes the results obtained in the *in vitro* bioassays with the plant extracts. Phylogenetic distribution of reported anti-CD plant taxa and screening results is shown in Figure 2. In general, the *T. b. brucei* strain was very sensitive toward the tested extracts, and, rather surprisingly, 90 extracts (78%) exhibited antitrypanosomal activity against procyclic *T. b. brucei*. In contrast, only 20 (17%) extracts inhibited *T. cruzi* epimastigotes with IC_{50} values $\leq$25 μg/mL. Seven extracts (6%) showed pronounced (IC_{50} < 10 μg/mL) and 13 extracts (10%) good ($IC_{50} \leq$ 25 μg/mL) antitrypanosomal activity.

The most potent extracts were those of *Cynara* sp. aerial parts (2 μg/mL), *Acanthostyles buniifolius* aerial parts (2 μg/mL), and *Gochnatia boliviana* leaves (4 μg/mL). The extracts from roots and flowers of *S. chloroclada*, the most frequently cited anti-CD botanical drugs among the Guaraní, were not active against epimastigotes (IC_{50} > 50 μg/mL), but an extract of the aerial parts of this plant was moderately active. The *S. chloroclada* flower extract showed significant inhibitory effects on parasite release in the

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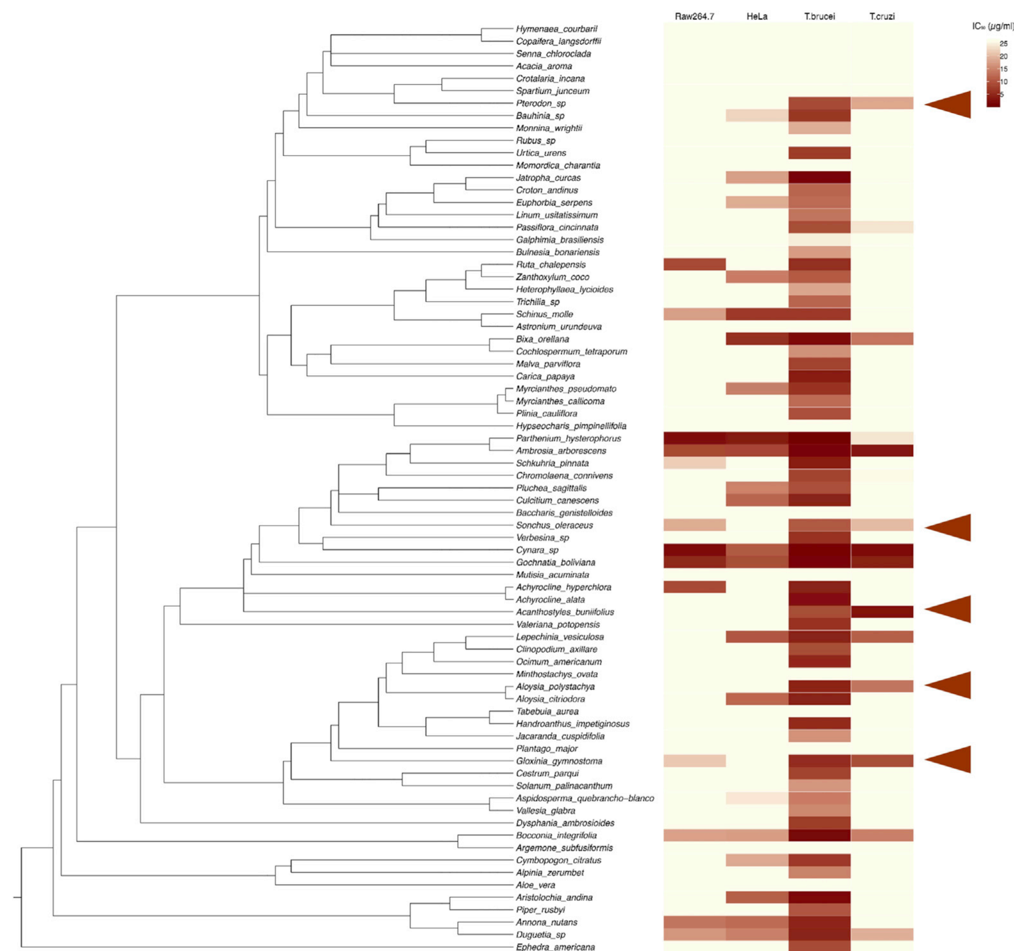

Figure 2. Biological profiling of the CD botanical drug library collected in Bolivia

Few EtOAc extracts showed selective toxicity toward *T. cruzi* epimastigotes (Y strain) with IC_{50} values below 20 μg/mL (arrows). A high number of extracts were toxic for procyclic *T. brucei*, and many showed antiproliferative effects in HeLa and Raw 264.7 cells. The Leguminosae was the only subfamily cluster (top) showing no activities up to 25 μg/mL. In the sesquiterpene lactone-rich family Asteraceae, only *Acanthostyles buniifolius* showed selective antitrypanosomal effects. Data represent profiling values (based on IC_{50} values) from at least two independent screening assays, each performed in triplicates. See also Table S1 and Figure S2.

infection assay (15 μg/mL was equally effective as 20 μM of benznidazole) without being cytotoxic to host cells (Table S1). A preliminary assessment of its phytochemical content was thus performed (*vide infra*). Since many extracts exhibited significant general cytotoxicity, the observed toxicity against epimastigotes could also be due to non-specific effects. Besides, cytotoxic extracts (27.8%) could not be analyzed in the trypomastigote release assay as the response could be erroneous due to the toxicity on the host cells. Particularly, cytotoxic extracts were those from *Ambrosia arborescens* leaves, *G. boliviana* leaves, *G. boliviana* branches, *Bocconia integrifolia* leaves, *B. integrifolia* roots, *Cynara* sp. aerial parts, and *P. hysterophorus* aerial parts. The only highly active and selective antitrypanosomal extracts were from *Pterodon* sp. seeds, *Sonchus oleraceus* leaves, *A. buniifolius* aerial parts, *Aloysia polystachya* aerial parts, and *Gloxinia gymnostoma* aerial parts (Figure 2). The extracts of these taxa also showed significant inhibitory effects in the *T. cruzi* infection assay (Table S1).

As initial IC_{50} values were obtained in antiproliferation assays with *T. cruzi* epimastigotes, we next screened the extracts lacking general cytotoxicity in the cellular infection assay. In order to validate the antitrypanosomal activity on the mammalian stage forms (trypomastigotes) that are relevant for the disease, extracts were tested for their ability to inhibit *T. cruzi* parasite release from infected chinese hamster ovarian cells (CHO). To that aim, we employed a versatile fluorescence-activated cell scanning (FACS)-based assay to

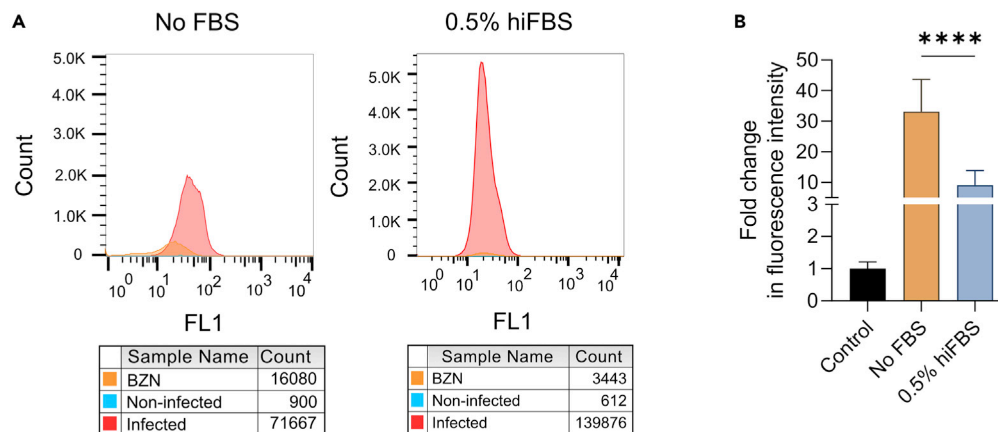

Figure 3. FACS parasite release assay in 0.5% FBS and serum-free host cell conditions

(A) Representative FACS histograms of the FL-1 channel (488/530 nm) showing parasites released into the medium from host cells in no FBS and 0.5% hiFBS cells infected with wild-type trypomastigotes. The vehicle control, benznidazole (BZN) treatment at 20 μM, and no infection control are shown. All samples were stained with the SYTO9 dye. Data are representative of at least six independent experiments.

(B) Measurement of reactive oxygen species in CHO-K1 cells cultured in the different FBS conditions. ROS levels were measured using the indicator DCFDA by FACS after 24 hr of exposure to different medium conditions. Control cells were cultured in complete medium. Bar graphs represent the mean fold change in the geometric mean of fluorescence intensity ±SD and were analyzed using FlowJo from three independent experiments performed in triplicate. Statistical significance was calculated with t test. ****$P < 0.0001$.

quantify the number of *T. cruzi* trypomastigotes released (and residual amastigotes in case cells burst prematurely). Using the SYTO9 DNA dye, the released parasites in the supernatant were stained (Figure 3A). Using culturing conditions without heat-inactivated fetal bovine serum (hiFBS) yielded consistently viable host cells with high reactive oxygen species (ROS) but reduced (about 50%) parasite release compared to CHO cells cultured in 0.5% hiFBS (Figures 3A and 3B).

Serum deprivation is known to increase intracellular ROS (Halliwell, 2003; Tangtrongsup and Kisiday, 2017) and helped to prevent overconfluency of the host cell layers over 6 days of culturing. Dichlorofluorescein diacetate (DCFDA) fluorescence was used to assess the relative amount of ROS during the critical infection time (24 hr). Host cells infected and treated in the presence of 0.5% hiFBS generated a lower level of ROS than cells cultured in the absence of hiFBS (Figure 3B). Amastigotes develop intracellularly, differentiate into trypomastigotes, and leave the host cell. As elaborated in the discussion, ROS is a factor in host cells that is important for parasite infection *in vivo*. Benznidazole was used to validate this parasite infection assay. Although the assay showed significant variability (±35%), due to the nature of independent *T. cruzi* infections, reliable IC_{50} values for benznidazole were obtained (in the range of 5–9 μM), thus somewhat higher than in previously published data (Da Silva et al., 2011; Koovits et al., 2020) Nevertheless, we employed this serum-free condition as it mimicked more closely the pathophysiological condition (see discussion) for our profiling of the CD botanical drug library. The initial testing of the plant extracts was performed at a single concentration (15 μg/mL). As shown in Table S1, extracts which were active against *T. cruzi* epimastigotes were generally also active in the infection assay. The pre-screening with epimastigotes was thus considered suitable for discovering antichagasic compounds able to inhibit different stages of the infection/replication cycle.

Comparative profiling of the DMM botanical drug library from the Mediterranean

To validate or challenge the ethno-directed approach for bioprospecting antichagasic activity, we compared the results obtained for botanical drugs used in a CD context with those of 660 botanical drugs described in Dioscorides' *DMM*. Notably, the botanical drugs mentioned in *DMM* have no traditional association with CD. EtOAc extracts were tested at 25 μg/mL *in vitro* against *T. cruzi* epimastigotes and on HeLa cells for cytotoxicity (Figure 4 and Table S2). The extracts were considered active (i.e., hits) when the percentage of inhibition was ≥50% at 25 μg/mL. A total of 59 (8.9%) extracts exhibited antitrypanosomal activity, while 102 (15.5%) extracts were cytotoxic toward HeLa cells. Among the antitrypanosomal hits, only 27 extracts (4.1%) were selectively toxic for *T. cruzi* epimastigotes over HeLa cells. Selective

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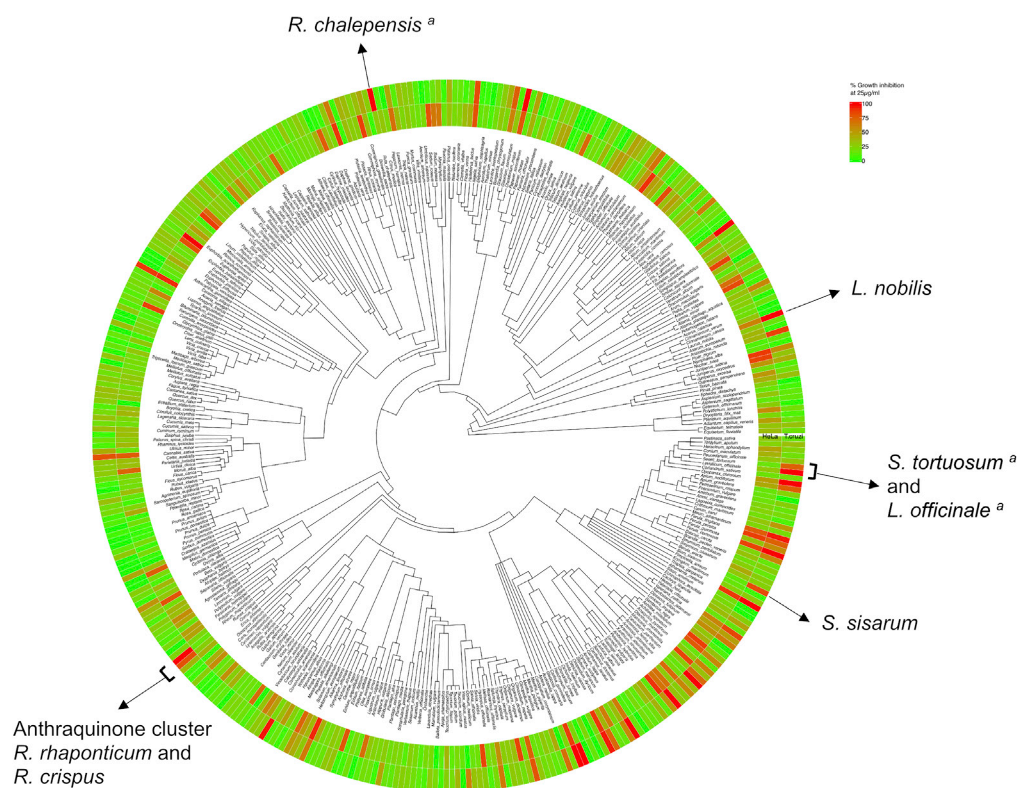

Figure 4. Comparative profiling of the DMM library from the Mediterranean

Phylobioactivity tree displaying phylogenetic relationships associated with bioactivities of EtOAc extracts. The outer ring shows growth inhibition on *T. cruzi* epimastigotes, and the inner ring shows growth inhibition of HeLa cells (both at 25 μg/mL; only most active plant part shown). A hypothetical coumarin cluster ([a]*Ruta chalepensis root*, *Levisticum officinale seeds*, and *Seseli tortuosum* root) and the anthraquinone cluster (*Rumex crispus* and *Rheum rhaponticum* rhizoma) are visible. *Laurus nobilis* root and fruits and *Sium sisarum* root (microfractionated) are indicated in the phylogenic tree. Detailed data on plant species and activities are shown in Table S2.

antitrypanosomal extracts were those obtained from *Levisticum officinale* fruits, *Opopanax chironium* roots, *Glebionis coronaria* flowers, *Tanacetum parthenium* flowers, *Convolvulus scammonia* roots, *Iris foetidissima* seeds, *Laurus nobilis* fruits, *Rheum rhaponticum* roots, *Rumex crispus* roots, and *Ruta chalepensis* roots. Extracts with selective toxicity for *T. cruzi* epimastigotes were considered potential hits, and their antichagasic activity was subsequently determined at 15 μg/mL in the trypomastigote release assay in CHO cells. All extracts except those obtained from *A. vera* resin, *L. nobilis* leaves, and *L. albus* roots were also active in the infection assay (>50% inhibition of parasite release at 15 μg/mL).

Comparison of extract libraries derived from disparate ethnopharmacological contexts

In an attempt to pharmacologically validate the ethno-directed approach, hit rates were calculated for both the Bolivian CD and Mediterranean *DMM* botanical drug libraries. The Pearson X^2 test was applied for statistics. Results show that there is a significantly higher probability (17.4% vs. 8.9%, $P = 0.0057$) of detecting antichagasic (*T. cruzi* epimastigotes) extracts when the plant had a reported use against CD. However, we also found a significantly higher percentage of cytotoxic ($IC_{50} \leq 25$ μg/mL) extracts among CD botanical drugs (27.8% vs. 15.5%, $P = 0.0012$). Taking the importance of selectivity into account, the hit rate of selective antichagasic extracts *in vitro* was not considered statistically different ($P = 0.079$) between the Bolivian CD (7.8%) and the *DMM* (4.1%) extract libraries. The two libraries shared 20 genera and the species *S. oleraceus*, *Spartium junceum*, *A. vera*, *Linum usitatissimum*, and *R. chalepensis* (Tables S1 and S2). Only *S. oleraceus* and *S. junceum* (both aerial parts) share also the botanical drug.

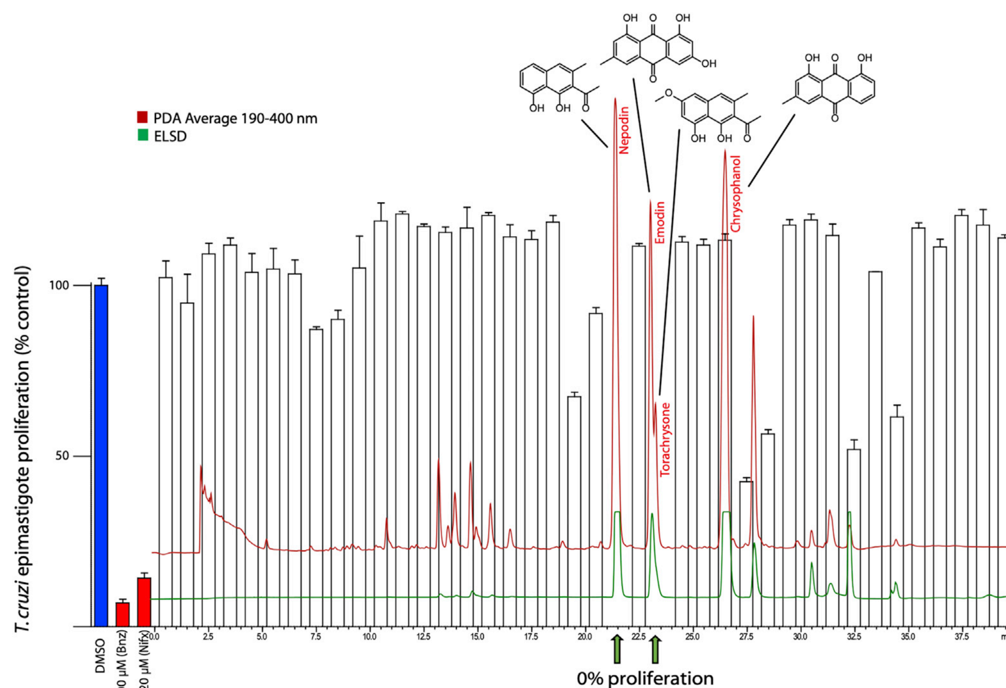

Figure 5. Microfractionation of selected extracts and identification of antitrypanosomal natural products

Bioactivity-guided microfractionation is exemplified with *R. crispus* using liquid chromatography and photodiode array (PDA) and evaporative light scattering detectors (ELSD). Isolation of antichagasic metabolites was based on epimastigote proliferation inhibition. False negatives are a limitation of this qualitative approach (shown here with chrysophanol) due to low concentrations. Nepodin and emodin/torachrysone were identified and isolated from fully active fractions (0% cell viability). The moderately active chrysophanol was identified in a negative fraction. Controls: BZN, benznidazole; Nifx, nifurtimox.

Microfractionation of selected extracts and identification of antitrypanosomal natural products

For the isolation of potentially antichagasic metabolites related to the *DMM* library, active extracts from different phylogenetic clusters (Figure 4) were selected. A major phylogenetic hotspot showing trypanocidal selectivity was the "anthraquinone cluster" with *R. crispus* (curly dock) and *R. rhaponticum* (rhapontic rhubarb) rhizomes from the Polygonaceae family. As anticipated, anthraquinones and naphthoquinones were major active principles in *R. crispus* (Figure 5) and *R. rhaponticum* (not shown). The latter also yielded the sesquiterpene lactone (SL) (6*R*, 7*S*)-costunolide. Since sesquiterpene lactones are known to be generally cytotoxic (Schmidt et al., 2009), we performed the host cell cytotoxicity assays also in serum-free conditions (Table 3). The observed differences (CC_{50} values) in serum-free conditions versus 0.5% hiFBS culture conditions likely resulted from differences in cell proliferation.

As illustrated with the *R. crispus* extract, the most potent antichagasic principle was the anthraquinone emodin, which completely inhibited *T. cruzi* epimastigote proliferation during the bioactivity-guided isolation (Figure 5). Emodin subsequently also inhibited parasite release *in vitro* with an IC_{50} value of 0.72 μM in the serum-free conditions but less potently in the presence of 0.5% hiFBS (IC_{50} = 9.52 μM) (*vide infra*). Nepodin from *R. crispus* inhibited epimastigote proliferation (IC_{50} = 28.7 $\pm$ 13.3 μM) and inhibited the parasite release in the infection assay (approximately 30% inhibition at 5 μM) (Table 3).

Taking the anthraquinone phylobioactive hotspot (Figure 4) and the strong ethnopharmacological consensus for *S. chloroclada* (*vide supra*) into consideration, we did a preliminary profiling of the anthraquinone content of this taxon. The presence of the anthraquinones emodin, aloe-emodin, and chrysophanol was confirmed by thin-layer chromatography (TLC) using reference compounds and Borntrager's test, as well as with an electrospray ionization mass spectrometry (ESI-MS) scan of the *S. chloroclada* aerial part extract (Figure S2).

Table 3. *In vitro* antiproliferative activity (50% inhibition [IC_{50}]) of compounds isolated from plant extracts on *T. cruzi* epimastigote stage (72 hr) and trypomastigote release (6 dpi)

Isolated cpd	IC_{50} epimastigotes [μM]	Percentage inhibition of parasite release (serum-free) at 5 μM	Cytotoxicity CC_{50} CHO cells [μM]	Cytotoxicity CC_{50} CHO cells without FBS [μM]
Nepodin[a]	28.7 ± 13.3	34.7 ± 30.9	>100	n.d
Torachrysone[a]	>50	25.9 ± 15.5	>100	n.d
Emodin[a]	14.1 ± 8.2	61.5 ± 18.2	>100	n.d
Falcarindiol[b]	>50	0	>100	n.d
Costunolide[c]	7.4 ± 5.9	0	10.9 ± 3.7	35.4 ± 13.8
Reynosin[d]	>50	50.0 ± 47.1	31.3 ± 8.0	66.1 ± 1.2
Santamarine[d]	19.5 ± 9.3	55.9 ± 46.7	13.2 ± 4.3	39.4 ± 1.5
Zaluzanin C[d]	6.7 ± 0.7	71.0 ± 7.5	6.6 ± 1.7	19.2 ± 5.2
3-Acetylzaluzanin C[d]	6.3 ± 0.9	83.0 ± 10.1	6.9 ± 2.4	n.d
Dehydrocostus lactone[d]	1.4 ± 0.4	86.2 ± 4.1	5.8 ± 1.9	12.2 ± 0.5
Eremanthin[d]	1.9 ± 0.3	87.6 ± 9.9	7.3 ± 1.4	11.5 ± 0.2
BZN (20 μM)	13.8 ± 2.9	76.9 ± 15.2	>100	n.d

Cytotoxic (antiproliferative) effects of the compounds were assessed on CHO host cells after 72 hr (50% cytotoxic concentration [CC_{50}]). Data shown are mean values ±SD of at least three independent experiments, each performed in triplicates. n.d, not determined; BZN, benznidazole.
[a]*Rumex crispus* root.
[b]*Sium sisarum* root.
[c]*Rheum rhaponticum* root.
[d]*Laurus nobilis* leaf.

Impact of 9,10-anthracenedione substitutions on antichagasic effects *in vitro*

Emodin (**2**), representative of the anthraquinone cluster, moderately inhibited *T. cruzi* epimastigote growth but significantly inhibited parasite release in the cellular infection assay (IC_{50} = 0.72 μM) without being cytotoxic to host cells up to 100 μM (selectivity index [SI] >7 (Table 5)). As shown below, emodin was less potent in the 0.5% hiFBS (low ROS) host cell culture conditions. In order to reduce the variability of the DNA staining and occasional cellular debris and to have a more versatile assay allowing tracking of amastigotes in follow-up experiments, we generated a green fluorescent protein (GFP)-expressing *T. cruzi* (Y) strain (see transparent methods). As shown in Figure 6A, the number of released trypomastigotes quantified was comparable to the wild type (Figure 3A). Moreover, the IC_{50} values obtained for benznidazole with the GFP-expressing parasites were not different from those of the SYTO9 staining on wild-type parasites. Intriguingly, benznidazole was significantly less potent to inhibit parasite release from host cells with high ROS, cultured without fetal bovine serum (FBS) (IC_{50} = 7.5 μM [95% confidence interval {CI} = 6.2–9.0 μM]) as compared to host cells cultured in 0.5% hiFBS with lower ROS (IC_{50} = 1.4 μM [95% CI = 1.1–1.6 μM]) (Figure 6B).

Using the GFP *T. cruzi* assay, we performed a preliminary SAR study on the 9,10-anthracenedione (**1**) scaffold (Table 4). The anthraquinones were screened for general cytotoxicity on CHO cells, epimastigote proliferation, and trypomastigote release from infected CHO cells (Table 5). For the *T. cruzi* parasite release assay, GFP-expressing trypomastigotes were used to infect CHO cells cultured with 0.5% hiFBS in the medium. Under these low ROS conditions, the IC_{50} of emodin was found to be significantly less potent than in the serum-free (high ROS) conditions used in the screening, namely 9.5 μM (95% CI = 8.2–10.9 μM) as compared to 0.7 μM (95% CI = 0.5–1.0 μM) (Figure 7A). Thus, emodin behaved in the opposite manner as benznidazole and was more than ten times less active in the low ROS host cell conditions (0.5% hiFBS). In the low ROS conditions, all active hydroxylated anthraquinones behaved in a similar manner showing *in vitro* antichagasic IC_{50} between 5 and 10 μM (Figure 7B).

Our profiling data indicate that the position of the hydroxyl groups was important for the antichagasic activity *in vitro*, with the trihydroxy-substituted derivatives being the most active (Table 5). The canonical

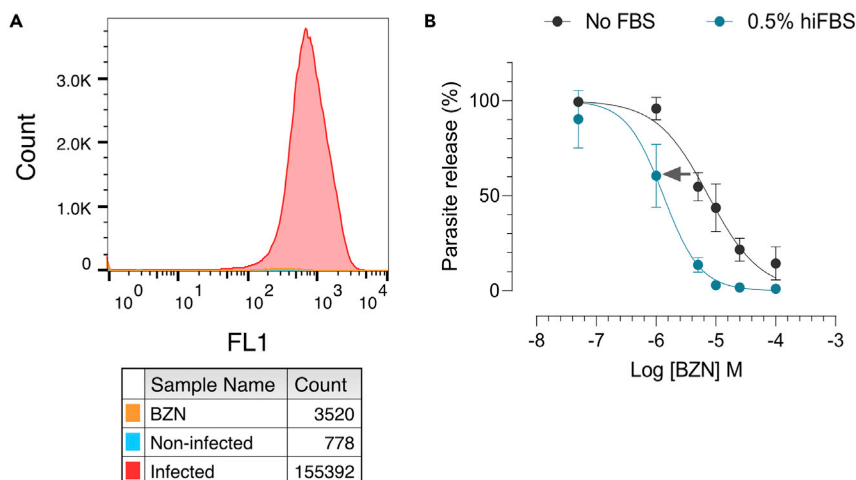

Figure 6. FACS parasite release assay using the GFP-expressing *T. cruzi* strain and reduced potency of benznidazole in serum-free culture conditions

(A) Representative FACS histograms of the FL1 channel (488/530 nm) showing parasites released into the medium from host cells in 0.5% hiFBS cells infected with GFP-expressing trypomastigotes. The samples were prepared by fixing the released trypomastigotes in 4% paraformaldehyde as described in transparent methods. The vehicle control, benznidazole (BZN) treatment at 20 μM, and no infection control are shown. Data show mean values ± SD of at least 6 independent experiments.

(B) Dose-dependent inhibition of parasite release by BZN under low and high ROS conditions. Data show mean values ±SD of at least three independent experiments performed in triplicate. See also Figure S3.

anthraquinone (**1**) was ineffective against epimastigotes and only marginally active against cellular parasite release at 5 μM. Hydroxyl groups at R1/R2 (**4, 5**) abolished this activity. Hydroxyl groups at R2/R6 (**9**) did not improve the activity of **1** in the infection assay. Likewise, the additional hydroxymethyl moiety at R3 found in aloe-emodin (**11**) increased the general cytotoxicity and the compound was found to have a selectivity index (SI) of 2.9.

Interestingly, hydroxylation at R1/R8 showed a trend toward a general increase in toxicity toward epimastigotes (**2**, **8**, **10**-**12**). The carboxylic acid at R2 abolished the antichagasic activity as indicated by the lack of activity of rhein (**15**) and the clinically used antirheumatic prodrug diacerein (**16**). The replacement of hydroxyl groups with methoxy and methyl groups generally led to decreased activity, as shown by compounds **12** and **18**. Thus, 9,10-anthracenedione with hydroxyl groups at positions R1 and R3 or R4, as exemplified by compounds **2**, **3**, and **6**, proved to be most active in the infection assay but did not inhibit proliferation of epimastigotes. We performed dose-response experiments for compounds that inhibited the parasite release by more than 25%, i.e., **3**, **6**, **11**, and **17** (Figure 7B). We also determined the long-term cytotoxicity (72-hr incubation) toward host cells for the anthraquinones that effectively inhibited *T. cruzi* parasite release in CHO cells (**1**, **2**, **3**, **6**, **9**, **10**, **11**, **14**, **17**). Noteworthy, all anthraquinones were cytotoxic only at high micromolar concentrations, with the exception of quinizarin, which showed a CC_{50} value of 38 ± 16 μM. In this study, emodin (**2**) was the most active antichagasic 9,10-anthracenedione in all stages of *T. cruzi in vitro,* followed by aloe-emodin (**11**), purpurin (**3**), and quinizarin (**6**).

DISCUSSION

Biological profiling of plant extracts and assessment of the ethno-directed approach

In the present study, we document the current knowledge about botanical drugs used to manage CD by the indigenous peoples Ayoreo, Chiquitano, Izoceño-Guaraní, and Quechua in Bolivia. A major aim was to use this information to validate the antichagasic ethnomedical plant resources present among the indigenous groups most affected by CD in Bolivia. Likewise, the multidimensional profiling allowed bioprospecting of anti-CD botanical drugs to inspire ethnopharmacologically driven drug discovery. Previous studies reported superior antimicrobial properties of extracts derived from botanical drugs selected based on popular uses related to microbial infections (Khafagi and Dewedar, 2000; Svetaz et al., 2010; Silva et al., 2013). Such studies lend credit to the ethno-directed approach in bioprospecting for specific bioactive

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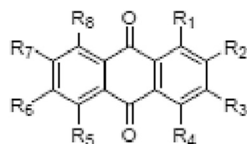
Table 4. Chemical structures of natural and synthetic anthraquinones tested on *T. cruzi*



ID		R1	R2	R3	R4	R5	R6	R7	R8
1	Anthraquinone	H	H	H	H	H	H	H	H
2	Emodin	OH	H	OH	H	H	CH_3	H	OH
3	Purpurin	OH	OH	H	OH	H	H	H	H
4	Alizarin	OH	OH	H	H	H	H	H	H
5	Alizarin Red S	OH	OH	SO_3Na	H	H	H	H	H
6	Quinizarin	OH	H	H	OH	H	H	H	H
7	Anthrarufin	OH	H	H	H	OH	H	H	H
8	Dantron	OH	H	H	H	H	H	H	OH
9	Anthraflavic acid	H	OH	H	H	H	OH	H	H
10	Chrysophanol	OH	H	CH_3	H	H	H	H	OH
11	Aloe-emodin	OH	H	CH_2OH	H	H	H	H	OH
12	Physcion	OH	H	OCH_3	H	H	CH_3	H	OH
13	2-Hydroxy-3-methylanthraquinone	H	OH	CH_3	H	H	H	H	H
14	2-Hydroxy-1-methylanthraquinone	CH_3	OH	H	H	H	H	H	H
15	Rhein	H	COOH	H	OH	OH	H	H	H
16	Diacerein	H	COOH	H	$O(C=O)CH_3$	$O(C=O)CH_3$	H	H	H
17	Disperse Red11	NH_2	OCH_3	H	NH_2	H	H	H	H
18	Aurantio-obtusin	OH	OCH_3	OH	H	H	CH_3	OH	OCH_3

metabolites. In order to challenge this approach, we tested a total of 775 EtOAc extracts from two independent botanical drug libraries generated from 79 taxa selected for their reported use against symptoms of CD in Bolivia and from 389 taxa described in *DMM*. Our findings indicate that the CD botanical drug library contains a significantly higher percentage of cytotoxic plant taxa. However, hit rates for selective antichagasic plant extracts in the two libraries were not significantly different. The overall higher hit rate of the CD library was possibly due to non-specific cytotoxic effects, which could be conditioned by the ecological factors prevailing in the Chaco and inter-Andean valleys. The extreme atmospheric and ecological conditions (altitude, extreme dryness, and high-temperature ranges) in these regions may favor the production of metabolites with broad-spectrum toxic or general antifeedant properties.

Among the indigenous peoples participating in this study, the biomedical concept of CD was only recently introduced and did not match with any existing traditional disease concept. This seems related to the fact that infection with *T. cruzi* shows a diffuse and varied disease pattern and is asymptomatic in most cases. We found that most botanical drug preparations intended for oral administration (aqueous decoctions and infusions) were used to relieve symptoms associated with the chronic phase of CD, such as cardiac complications, fever, and fatigue, and not for combatting the (invisible) parasites. In fact, most of the botanical drugs applied for CD-related symptoms were also used for other therapeutic purposes involving inflammatory conditions (Table 2). We thus conclude that tangible ethnomedical concepts about CD were absent until recently and developed only during the last decades, which is in agreement with the cultural perception of *T. cruzi* vectors (Salm and Gertsch, 2019). This clearly hampers the application and selection of botanical drugs targeting *T. cruzi* parasitemia and its symptoms and probably explains in part why the majority (>80%) of the extracts derived from botanical drugs with reported use against CD and its symptoms were not active. The use of plant- and animal-based traditional medicine among the Chiquitano, Izoceño-Guaraní, and Quechua was widespread and in agreement with

Table 5. *In vitro* antiproliferative activity of anthraquinones on *T. cruzi* epimastigotes (72 hr) and GFP-expressing trypomastigotes release (6 dpi)

ID	IC_{50} epimastigotes[a] [μM]	Percentage inhibition of parasite release[b] at 5 μM (low ROS)	CC_{50} CHO cells [μM]	Selectivity Index (CC_{50} CHO/IC_{50} Epi)
1	>50	22.2 ± 9.6	>100	n.d
2	14.1 ± 8.1	24.8 ± 12.9	>100	>7
3	>50	48.3 ± 18.1	59.5 ± 10.1	n.d.
4	>50	0	n.d.	n.d.
5	>50	0	n.d.	n.d.
6	>50	68.5 ± 19.2	38.1 ± 16.0	<1
7	>50	0	n.d.	n.d.
8	5.3 ± 2.3	0	n.d.	n.d.
9	>50	0	n.d.	n.d.
10	6.6 ± 3.5	22.6 ± 17.2	35.7 ± 7.4	5.4
11	14.5 ± 5.6	44.0 ± 17.6	42.0 ± 15.5	2.9
12	6.4 ± 3.7	0	n.d.	n.d.
13	>50	0	n.d.	n.d.
14	>50	20.9 ± 9.7	88.9 ± 8.9	n.d.
15	>50	0	n.d.	n.d.
16	>50	0	n.d.	n.d.
17	32.9 ± 13.9	46.8 ± 25.6	>100	>3
18	>50	0	n.d.	n.d.
BZN	13.8 ± 2.9	84.9 ± 4.8	>100	>7

Compounds that inhibited >20% parasite release in the trypomastigote infection assay at a single concentration of 5 μM when cultured with 0.5% hiFBS were tested for antiproliferative effects on CHO host cells. Data shown are mean values ±SD of at least three independent experiments, each performed in triplicates. n.d., not determined; BZN, benznidazole.

[a]IC_{50} values of the compounds against epimastigotes were assessed as described in the transparent methods.

[b]The percentage inhibition of parasite release by the compounds was estimated at a single concentration (5 μM) as described in transparent methods.

previous reports that these people widely use traditional medicine despite the presence of Western health care (Quiroga and Arrázola, 2013; Vandebroek et al., 2008). Noteworthy, the Ayoreo did not treat CD at all, which agrees with their overall minimal use of herbal medicine. Chiquitano, Izoceño-Guaraní, and Quechua research participants stated that they tried to manage CD with plant-based remedies because Western health care was limited and chemotherapy not accessible during the chronic stage of CD. Another study from a different region in Bolivia reported a similar situation (Forsyth, 2017). Asteraceae was the most dominant family of plants used for CD, likely due to their overall abundance and species richness. In general, Asteraceae is over-proportionally represented in medical florae (Heinrich et al., 1998; Moerman et al., 1999; Thomas et al., 2009), and this may be linked to the high diversity of bioactive secondary metabolites in the family (Heinrich et al., 1998). Quechua informants showed a low consensus regarding the species to be used in the treatment of CD, with only one species mentioned three times. A higher consensus was found among the Chiquitano and Izoceño-Guaraní participants (estimated Trotter and Logan Informants' consensus: Fic >0.8), with relatively few species being used by a large proportion of participants. Among the Izoceño-Guaraní, *S. chloroclada* (referred to as lanza lanza, mbuijare, or retama) was clearly the most important species for treating CD, with a share of 48% of total use reports. Interestingly, *S. chloroclada* belonged to a phylogenetic cluster that showed no or little inhibition of epimastigote proliferation in the pre-screening (Figure 2) but exhibited significant *in vitro* antichagasic effects in the parasite release assay (79.5% inhibition at 15 μg/mL by EtOAc extract of the flower). This discrepancy was also observed with some anthraquinones (purpurin and quinizarin). Since the extract likely contains glycosides that may be hydrolyzed by CHO cells but not by epimastigotes, we cannot exclude metabolic changes induced by host cells. The genus *Senna* (syn. *Cassia*)

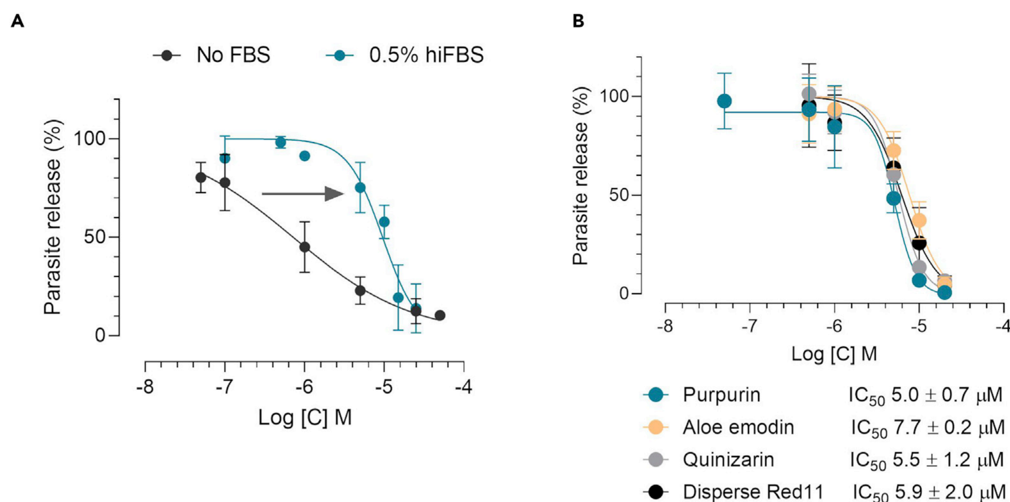


Figure 7. Emodin potency is dependent on serum and host cell ROS concentration

(A) Dose-dependent inhibition of parasite release by emodin under serum and no serum (low and high ROS) conditions.
(B) Dose-dependent inhibition of parasite release by hydroxyanthraquinones which inhibited more than 25% release at 5 μM using 0.5% hiFBS (low ROS): purpurin, aloe-emodin, quinizarin, and disperse Red11. Data represent the average ± SD of three independent experiments each performed in triplicate.

is known to contain anthraquinone, dianthrone, and naphthol glycosides (Dave and Ledwani, 2012). A preliminary ESI-MS scan and TLC confirmed the presence of anthraquinones in this botanical drug (Figure S2). It may not be a coincidence that the anthraquinone phylobioactive hotspot includes *S. chloroclada* which was one of the most frequently used taxa for CD. In fact, *S. chloroclada* flowers and roots are the only botanical drug repetitively stated to treat CD among the Izoceño-Guaraní. To date, no in-depth phytochemical study is available on *S. chloroclada*, and follow-up studies are planned. *T. aurea* bark, as its Spanish vernacular name "paratodo" indicates, was used for numerous diseases, in agreement with a previous study (Hajdu and Hohmann, 2012). The introduced species *A. zerumbet* and *C. citratus* are well known in South America for the treatment of cardiovascular diseases (Hajdu and Hohmann, 2012; Lim, 2016; Luz et al., 1984; Moreira et al., 2010). An apparently specific medicinal indication for CD was also reported for *Aloysia citrodora*, *Baccharis genistelloides*, *Bixa orellana*, *Dysphania ambrosioides*, *Handroanthus impetiginosus*, *L. usitatissimum*, *Plantago major*, *Ruta chalepensis*, *S. junceum*, and *Schinus molle* (Alonso, 2000; Bastien, 1998; Bourdy et al., 2004; Forsyth, 2017; Grandi et al., 1989; Martínez et al., 2004; Martino, 2012; Quiroga et al., 2012; Report, 2002); . None of the EtOAc extracts obtained from botanical drugs of these species were significantly and selectively toxic against *T. cruzi* in our infection assay with the exception of *S. chloroclada* (flowers) inhibiting parasite release >50% *in vitro* at 15 μg/mL (Table S1) but not showing any effect on epimastigotes.

The extract obtained from the aerial parts of *A. buniifolius* had an SI > 20 and was the other noteworthy hit obtained with the CD-informed library. It fully inhibited epimastigote proliferation at 25 μg/mL and parasite release at 15 μg/mL. Its antichagasic and antileishmanial activity was previously reported for plant material collected in Argentina, and the flavonoid santin was thought to be the active compound (Sülsen et al., 2007). However, santin was only moderately active against *T. cruzi* epimastigotes and trypomastigotes (IC_{50} values >30 μM). Certainly, the presence of additional antichagasic metabolites should not be excluded, and an in-depth phytochemical investigation of *A. buniifolius* is warranted. The extract of seeds of a *Pterodon* sp. showed significant and specific antitrypanosomal effects. Whether the very common diterpene alcohol geranylgeraniol, previously identified as the major antichagasic component in *Pterodon pubescens* seeds (Menna-Barreto et al., 2008), is responsible for this specific antitrypanosomal activity remains to be clarified.

The screening of the *DMM* extract library resulted in 23 extracts with selective parasite toxicity in the *T. cruzi* release assay. Of these extracts, eight were from the Apiaceae family and seven from the Asteraceae family showing taxonomic parallels with the CD-informed library. Asteraceae is the largest plant family and characterized by the presence of sesquiterpene lactones (SLs) several of which known to have antiprotozoal

properties (Schmidt et al., 2009; Sepúlveda-Robles et al., 2019) (*vide infra*). It is possible that the use of SL containing botanical drugs could therefore represent an ethnopharmacological strategy to reduce parasitemia in CD. The 660 extracts representing the *DMM* library were obtained from 389 different plant species. The largest part is from the Mediterranean basin, but central European species and exotic herbal drugs imported from Africa, Arabia, Central Asia, Himalaya, and the Indo-Malayan region are also included. The families with the highest share of botanical drugs and species are Apiaceae with 69 botanical drugs from 37 spp., Asteraceae with 51 botanical drugs from 33 spp., Rosaceae with 37 botanical drugs from 18 spp., Lamiaceae with 33 botanical drugs from 26 spp., and Fabaceae with 29 botanical drugs from 22 spp. This pattern reflects the overall taxonomic composition of *DMM* for which a total of 536 plant taxa representing 924 botanical drugs were identified and recommended for 5314 medical applications (Staub et al., 2016). The medical categories with the most therapeutic uses are dermatology (1216), gastroenterology (805), gynecology (615), urology (437), respiratory system (374), and neurology (269). The most frequently mentioned parasite treatments are related to lice, scabies, and tapeworms but also applications for malaria causing infections with *Plasmodium* were described (tertian and quartan fever). This includes parts of *Anchusa* sp., seed and leaves of *Bituminaria bituminosa*, root of *Dipsacus fullonum*, seeds of *Heliotropium europaeum*, root of *Plantago* sp., herb of *Potentilla reptans*, and the herb of *Verbena officinalis* (Staub et al., 2016). With respect to the overall Mediterranean flora, the Apiaceae and Rosaceae appear to be overrepresented in this library, while the frequency of Asteraceae and Lamiaceae appears to be rather consistent with the overall species diversity and abundance. Apiaceae fruits were frequently used as antidotes and their resins for neurological and musculoskeletal problems. Considering the size of plant families, the Fabaceae seem underrepresented, while Poaceae, Caryophyllaceae, and Orchidaceae are clearly underrepresented in the *DMM* library (Table S2 Staub et al., 2016; Tutin, 1978). Concerning the treatment of fevers and parasites, the active extracts from plants belonging to the "coumarin cluster" such as the herb of rue (*R. chalepensis*) were recommended in *DMM* for the internal use of the treatment of tremor and shivering before fever attacks occur, while the seeds of *Seseli tortuosum* are suggested against fevers in general. The hits belonging to the "anthraquinone cluster" such as the roots of *Rumex* species and rhubarb (*Rheum* spp.) were recommended for the treatments of scabies and fevers, respectively. For *L. officinale*, *L. nobilis*, and *S. sisarum*, no uses related to fever and parasites are recorded in *DMM* (Staub et al., 2016).

Microfractionation and isolation of bioactive principles of selected antichagasic plant taxa from the DMM library

For the isolation of active compounds from the *DMM* library, we selected extracts pertaining to three different phylogenetic groups, with taxa whose plant material was readily accessible. A major phylogenetic hotspot showing apparent antitrypanosomal selectivity was the anthraquinone cluster with the rhizomes of *R. crispus* (curly dock) and *R. rhaponticum* (rhapontic rhubarb) from the Polygonaceae family. The anthraquinones emodin and chrysophanol were identified in *R. crispus* and served as a basis for the subsequent preliminary SAR study (*vide infra*). Since the naphthoquinone derivative nepodin with known antimalarial activity (Alonso, 2000) was isolated together with anthraquinones from *R. crispus* and likewise inhibited epimastigote proliferation, the potency of the extract may reflect additive effects. In general, naphthoquinones have been studied extensively in *T. cruzi* epimastigotes (Salas, 2011; Ventura Pinto and Lisboa de Castro, 2009) without leading to a successful translation to clinical trials (Sales Junior et al., 2017). Extracts with antitrypanosomal activity from plants with distinct phylogenetic positions were those from *L. nobilis* (bay) (Lauraceae) and *S. sisarum* (skirret; Apiaceae). *L. nobilis* leaves contain a range of SLs, which may explain the significant antichagasic effects of its extract in epimastigotes. Numerous studies have addressed the selective versus non-selective antitrypanosomal effects of SLs, which are also widely present in Asteraceae (Kimani et al., 2018; Moraes Neto et al., 2019; Muschietti and Ulloa, 2016). SLs like cynaropicrin and others can act as electrophiles and form adducts with biological nucleophiles, such as trypanothione, the parasitic equivalent of glutathione in mammalian cells (Zimmermann et al., 2013, 2014). The reason why *L. nobilis* (leaves) was ineffective in the infection assay could be due to the SLs reacting with thiols in host cells, e.g., glutathione, without reaching the parasite. It has been shown that CHO cells can produce glutathione upon stress (Orellana et al., 2015). Thus, SLs undergoing a Michael-type addition with thiols are likely poorly bioavailable to infected tissues as they are detoxified by glutathione. Although the polyacetylene falcarindiol from *S. sisarum* had no inhibitory effect on *T. cruzi*, we cannot exclude the possibility that other polyacetylenes present in *S. sisarum* root may be more potent as indicated by the activity profile of the extract.

SAR study of anthraquinones as antichagasic natural products *in vitro* and importance of ROS in host cells

Anthraquinones are condensed aromatic hydrocarbons found in different plant species known for their medical and dye applications (Malik and Müller, 2016). In Western pharmacopeias, anthraquinone containing botanical drugs such as *Rhamnus* spp. (Frangulae cortex and Rhamni purshiani cortex) or *Rheum officinale* (Rhei radix) are used as laxatives. To elaborate on our natural product drug discovery approach, the most active antichagasic secondary metabolite emodin (1,3,8-trihydroxy-6-methylanthracene-9, 10-dione) led us to explore the SAR of differentially substituted 9,10-anthracenediones. Trihydroxylated anthraquinones have already been shown to be trypanocidal (*vide infra*). However, the present work provides a preliminary SAR study on 9,10-anthracenediones for both *T. cruzi* epimastigote and parasite release from trypomastigote-infected CHO cells. Different anthraquinones have been shown to exert moderate to good antimalarial, antibacterial, and antiviral effects *in vitro* at low micromolar concentrations (Li and Jiang, 2018). In our study, emodin showed specific inhibition of *T. cruzi* parasite release in CHO cells in the nanomolar or low micromolar range, depending on host cell culture condition. Emodin has been tested previously only on epimastigotes at high micromolar concentrations (De Lima et al., 2017) and as weak inhibitor of casein kinase 1 with an IC_{50} value of 130 μM (Justiniano et al., 2014). Other reported effects of emodin include anti-inflammatory, antiosteoporosis, anti-cardiovascular disease, and antidepressant effects (Li and Jiang, 2018). Purpurin, a natural tri-hydroxylated anthraquinone, inhibited *T. cruzi* parasite release and showed a selectivity index of 7. Rather unexpectedly, it did not inhibit epimastigote proliferation, which may be due to differences in pH between lysosomes and culture medium related to the trypanosomal uptake of anthraquinones. Purpurin has previously been shown to inhibit blood stream trypomastigotes (De Castro, Pinto and Pinto, 1994) but again has not been studied in cellular *T. cruzi* infection assays. The 9,10-anthracenedione scaffold is likely to interfere with the parasite redox system as anthraquinones can mediate the production of hydrogen peroxide or ROS via oxygen reduction *in situ* (Campos-Martin et al., 2006). This is a feasible antichagasic mechanism as anthraquinones have been shown to interfere with redox reactions in cells (Okumura et al., 2019). ROS generation is also the postulated mode of action of the approved antichagasic drug benznidazole (Pedrosa et al., 2001). Interestingly, based on the *T. cruzi* parasite release assay conducted using different serum conditions in combination with differential ROS amounts in host cells, it was evident that the generation of ROS plays a key role in the mechanisms of action of benznidazole and emodin with opposite effects. The IC_{50} value of benznidazole reduced 5-fold in the presence of only 0.5% hiFBS, whereas the IC_{50} of emodin increased by 13-fold, probably as a function of increased ROS. This striking difference could have translational implications for CD treatment, for instance, in the context of the lack of efficacy of benznidazole against residual amastigotes in advanced CD stages. Moreover, benznidazole emodin combinations may be tested for potential synergistic antichagasic effects *in vivo*. However, as reviewed recently, emodin shows relatively low oral bioavailability in rodents (5–10%) (Li et al., 2020). As some anthraquinones did not inhibit *T. cruzi* epimastigote proliferation in our study (Table 5), their antichagasic effects in the infection/parasite release assays may be due to host cell ROS generation induced by these compounds. Anthraquinones are primarily present in their glycosylated form in plants. Since anthraquinone glycosides can be deglycosylated and reduced to anthrones and anthranols by gut bacteria (Hattori et al., 1988), resulting in potent laxative effects, the systemic application of these compounds can be challenging. However, as exemplified by the clinical pharmacokinetics on diacerein (prodrug of rhein), low micromolar plasma concentrations can be achieved (Nicolas et al., 1998). Unfortunately, rhein showed no antichagasic effects in our assays, possibly due to the carboxylic acid at C2. It is noteworthy that the most important botanical drug used in the context of CD among the Guaraní, *S. chloroclada*, also contains anthraquinone aglyca, thus potentially rendering the anthraquinone cluster as relevant in the ethnomedical treatment of CD.

Overall, the comparative phylobioactivity-guided screening for *in vitro* antichagasic activity is an enabling tool to investigate plant-based ethnomedical resources and for the discovery of natural products that may have potential in CD drug development. Our study demonstrates the potential of plant secondary metabolites as prospective antichagasic principles in a comparative matrix, linking ethnomedical information with phylogenetics and chemotaxonomy. The comparative profiling also facilitated the pharmacological validation of the extract library from botanical drugs currently used in the context of CD in Bolivia, thus challenging the ethno-directed CD bioprospecting approach. We did not find statistical evidence that the CD botanical drug extract library yielded more hits than the *DMM* drug extract library. Nevertheless, our study led to the identification of significant antichagasic phylogenetic hotspots in the plant kingdom that may serve as a basis for future phytochemical investigations and antichagasic bioprospection.

Limitations of the study

Polar secondary metabolites like glycosides, polymers, or polyphenolics potentially present in the botanical drugs but generally showing poor systemic bioavailability were not present in the EtOAc extracts. In addition, the potential additive or antagonistic effects of natural products present in the plant extracts were not investigated. The *in vitro* profiling may give false-negative results as secondary metabolites are metabolized *in vivo* and can serve as inactive pro-drugs. We assessed the activity of the compounds and extracts using a validated FACS-assisted *T. cruzi* parasite release assay, which allowed us to perform relative quantifications of parasite load using appropriate controls. However, the method did not discriminate between amastigote replication and the release of the parasites from the host cells.

Resource availability

Lead contact

Further information and requests for resources should be addressed to and will be fulfilled by the lead contact Jürg Gertsch (gertsch@ibmm.unibe.ch).

Material availability

This study did not generate any unique reagents.

Data and code availability

The data supporting the findings of this study are available within the paper and its supplemental information.

METHODS

All methods can be found in the accompanying transparent methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102310.

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AUTHOR CONTRIBUTIONS

A.S., S.R.K., and J.G. conceived and managed the project. A.S. carried out the fieldwork, analyzed, and curated data under the supervision of J.G. G.A. enabled the access to herbaria and contributed logistically to the fieldwork. M.C. and M.L. assembled the Dioscorides extract library. A.S. and S.R.K. carried out *in vitro* experiments with *T. cruzi*. A.S. and M.C. profiled plant extracts on different cells. O.D. performed the bioactivity-guided isolation of natural products under the supervision of M.H. A.S. and S.R.K. established the FACS parasite release assay. S.R.K. performed the SAR analysis with anthraquinones. M.C. and A.S. assembled the phylogenetic trees. A.S. and J.G. wrote the manuscript with input from all the authors. All the authors edited the manuscript to its final form. J.G. supervised the project.

DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest.

INCLUSION AND DIVERSITY

For the ethnopharmacological fieldwork, we worked to ensure gender balance in the recruitment of human subjects, to ensure ethnic or other types of diversity in the recruitment of human subjects, and to ensure that the study questionnaires were prepared in an inclusive way. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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Supplemental information

Phylobioactive hotspots in plant resources used to treat Chagas disease

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Transparent Methods

Ethnopharmacological fieldwork

The study was carried out among four indigenous groups (Quechua, Izoceño-Guaraní, Chiquitano, and Ayoreo), settled in geographically distinct rural areas in Bolivia (Figure 1). The surveyed Quechua communities were located in the Inter-Andean valleys between 3000 and 2200 m.a.s.l. in the municipality of Mizque (Cochabamba Department). The Guaraní in Bolivia live in the Chaco region, which is a semi-arid plain grassland, interspersed with swamps and thorny forests, that extends across southeast lowland Bolivia. In Bolivia, there are three subgroups of Guaraní (Ava, Simba, and Izoceño), marked by linguistic and historical differences. We visited communities of the Izoceño-Guaraní in the municipality of Charagua (Santa Cruz Department). The Chiquitano informants lived in the lowland Chiquitanía region situated in the Santa Cruz Department. This region is an ecologically transitional zone between the arid plains of the Chaco and the tropical rain forests. Today, Chiquitanos are native Spanish speakers. The survey was carried out in the Chiquitano communities in the municipalities of Concepción, San José de Chiquitos, and Roboré. The Ayoreo groups used to live in the Chaco but have been sedentarized and acculturated by missionaries in the mid-twentieth century. We surveyed Ayoreo communities situated in the Chiquitanía region (municipalities of Concepción, Pailón, and San José de Chiquitos) and Ayoreo informants who lived in the city of Santa Cruz. All visited indigenous communities were small settlements located in rural areas characterized by a low population density. The main economic activity was subsistence farming. Traditional dwellings were predominantly constructed of adobe walls, earthen floors and thatched roofs, and constitute an ideal habitat for the CD vector *T. infestans* (Salm and Gertsch, 2019). *T. cruzi* transmission was hyperendemic, showing the highest infection rates in the Chaco region and Inter-Andean valleys (Brenière et al., 2002; Chippaux et al., 2008; Gürtler, 2009; Ministerio de Salud, 2015; Samuels et al., 2013). Biomedical health care was mainly provided by primary health centers located in the bigger villages Charagua, La Brecha and Iyovi (Chaco), San José de Chiquitos, Roboré and Concepción (Chiquitanía), Mizque, and Laguna Grande (Inter-Andean valleys). However, most of them were insufficiently equipped, and CD treatment was marginally successful (Salm and Gertsch, 2019). Additionally, a survey was conducted in the herbal markets of the cities of La Paz and Santa Cruz de la Sierra.

Fieldwork was carried out between June 2014 and May 2015 in all study sites during all vegetation periods. The survey was directed towards the management of CD related symptoms and associated knowledge. Before starting with the survey, the project was presented to the local authorities and the communities (Figure 1) during initial meetings for approval. Oral consent was obtained from each research participant for treating personal data and the recording of ethnomedical information. Local research participants were randomly selected according to their availability, willingness, and individual interest, while each participant represented a distinct household. Interviews took place at the participants' home and were conducted in Spanish or in their native language (Guaraní, Ayoreo, Quechua) with the assistance of a local translator. In the cities, we interviewed the medicinal plant vendors at the herbal markets from where we directly purchased the botanical drugs. Ethnomedical and ethnopharmacological information was obtained through structured and semi-structured interviews, free listing of remedies, observing practices *in situ,* and by collecting plant material during walk-in-the-woods together with the participants. During interviews, representative photographs of chagoma were shown, and symptoms of chronic CD (cardiomyopathy, megacolon, megaoesophagus) described to the participants for eliciting focused responses. Questions targeted ethnomedical concepts related to CD and plant taxa used to treat acute

and chronic symptoms. Vernacular plant names, parts used, dosage, mode of preparation and administration, provenance, and availability were documented. Botanical voucher specimens were collected with the help of local informants, prepared and deposited at the National Herbarium in Bolivia (LPB) under the collection ID ASMPx. Botanical identification was carried out by specialists at LPB, and botanical names standardized in accordance with the theplantlist.org ("The Plant List, Version 1.1," 2013) and plant families following Angiosperm Phylogeny Group 4 (APG IV, 2016) (The Angiosperm Phylogeny Group. et al., 2016). Data were analyzed with basic descriptive statistics such as the quantification of individual use reports cited by the informants according to current standards and recommendations (Weckerle et al., 2018).

Collection of botanical drugs mentioned in De Materia Medica

Botanical drugs mentioned in *De Materia Medica (DMM)* and associated voucher specimens were collected in several locations in Europe and the Mediterranean area, cultivated in domestic gardens or purchased from commercial suppliers between 2014 and 2016. Plant taxa were identified using the Flora Europea (Tutin et al., n.d.). Only those 660 botanical drugs, whose botanical description in *DMM* permitted a taxonomic identification (Staub et al., 2016) were collected. Before solvent extraction, samples were dried at 40–60 °C. Herbarium voucher specimens were identified at the Department of Biomedical Science (University of Cagliari) and voucher specimens deposited at the Herbarium of the Botanical Garden of Geneva (Switzerland) and the Herbarium of the National and Kapodistrian University of Athens (ATHU).

Preparation of extracts

Medicinal plant parts used in the context of CD were collected *in situ,* and from the same collections, herbarium voucher specimens were prepared. The clean plant materials (roots washed) were air-dried in the shade and stored dry in cotton bags at -20 °C prior to extraction. The extracts were generated as follows: Air-dried and powdered (0.5 mm) plant material was used for the preparation of semipolar extracts. The samples were exhaustively extracted by maceration with EtOAc (99.8%, Sigma Aldrich) at room temperature for 48 h. EtOAc (polarity index P = 4.4) was used based on its optimal extraction for secondary metabolites that are apolar to semipolar, excluding the highly polar constituents like sugars and polymers. Upon filtration (Whatman, 2.5 µm), samples were concentrated under reduced pressure on a rotary evaporator. The dried extracts were used to prepare stock solutions of 5 mg/mL in dry DMSO (≥99%, Sigma Aldrich) for subsequent use in *in vitro* assays. Crude extracts and DMSO stock solutions were stored at -20 °C.

Mammalian cell culture

Immortalized hamster ovary CHO-K1 (ATCC® CCL-61™), human cervical HeLa (ATCC® CCL-2™), and mouse macrophage Raw 264.7 (ATCC® TIB-71™) cells were cultured in RPMI-1640 medium supplemented with 10% FBS (fetal bovine serum) at 37 °C in 5% CO_2 atmosphere.

Parasite culture and metacyclogenesis

T. cruzi (Y strain obtained from ATCC) were maintained in liver infusion tryptose medium (LIT) supplemented with 10% heat-inactivated FBS at 27 °C, prepared according to the recommendations by ATCC. Metacyclogenesis of epimastigotes was induced by Grace insect medium (Sigma Aldrich, MO, USA)

supplemented with 10% FBS and hemin (25 μg/mL, Sigma Aldrich, MO, USA). Trypomastigotes were generated by infecting CHO cells with metacyclic trypomastigotes harvested after nine days of cultivation in Grace medium (Sullivan, 1982). Trypomastigotes of *T. cruzi* were obtained from the extracellular medium of infected CHO cells at day five post-infection. Alternatively, metacyclic trypomastigotes were also obtained by incubating stationary phase epimastigotes in triatomine artificial urine medium (TAU, 190 mM NaCl, 17 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 7.5 mM NaH_2PO_4, 0.5 mM Na_2HPO_4, pH 6.0) for 2h at a density of 5 x 10^8 cells/mL. After 2 h, the cell suspension was diluted in TAU3AAG (TAU supplemented with 10 mM L-proline, 50 mM sodium L-glutamate, 2 mM L-aspartate, and 10 mM glucose) to a concentration of 5 x 10^6 cells/mL and incubated at 28 °C for five days (Figueiredo et al., 2000).*T. b. brucei* procyclic form (427 strain) was cultured at 27 °C in SDM79 medium, supplemented with 5% FBS and hemin (Brun and Schonenberger, 1979).

Generation of GFP expressing T. cruzi

Epimastigote (Y strain) forms were grown to a density of approximately 10-20 x 10^6 cells/mL. The cells were washed with PBS and resuspended in Cytomix electroporation buffer (120 mM KCl, 0.15 mM $CaCl_2$, 10 mM K_2HPO_4, 25 mM HEPES, 2 mM EDTA, 5 mM $MgCl_2$, pH 7.6) at a density of 10^8 cells/mL (Lander et al., 2015). The cells were then transferred to 0.4 cm electroporation cuvette and 25 µg of pTREX-n-eGFP plasmid was added. pTREX-n-eGFP was a gift from Rick Tarleton (Addgene plasmid # 62544; http://n2t.net/addgene:62544; RRID:Addgene_62544) (Peng et al., 2015).The mixture was placed on ice for 10 min and then subjected to three pulses of 1.5 kV and 25 µF with a Gene Pulser II (Bio-Rad, Hercules, USA). The electroporated cells were allowed to rest for 15 min at room temperature until their transfer to 5 mL of LIT medium supplemented with 20% heat-inactivated FBS and incubated at 27 °C. After 24 h, 250 µg/mL of G418 (InvivoGen) was added to select for transfected parasites. During the selection process, the medium was replaced with fresh LIT medium supplemented with 20% FBS and antibiotic once a week for nearly 4-5 weeks. Once the parasites reached a density of 20 x 10^6 cell/mL, the cells were diluted and maintained in LIT medium supplemented with 10% FBS and 500 µg/mL of G418. Metacyclogenesis of transfected epimastigotes was performed using TAU medium, as mentioned above.

In vitro *activity against* T. cruzi *epimastigotes*

Antitrypanosomal activity was first evaluated against epimastigotes of *T. cruzi* by the XTT assay (Benabdelaziz et al., 2018; Salamanca Capusiri et al., 2008). Since all known antichagasic agents show toxicity also against the epimastigote form, we used this screening as a preselective assay. Epimastigotes in exponential growth phase were counted, adjusted to a concentration of 1.5 x 10^6 parasites/mL, and exposed to 6 concentrations of each plant extract (in DMSO) ranging from 100 μg/mL to 0.8 μg/mL for 72 h at 27 °C. For the *DMM* library we chose a concentration of 25 μg/mL based on recommendations for the pure compounds (Don and Ioset, 2014). The assays were carried out in 96-well plates (200 μL/well). After 72 h of incubation, the plates were inspected under an inverted microscope to assure the growth of the controls and sterile conditions. 50 μL of a XTT (Sigma Aldrich, MO, USA)/PMS (phenazine methosulfate, Sigma Aldrich, MO, USA) solution (0.5 mg XTT/0.025 mg PMS/mL in PBS) were added to the plates. The parasites were further incubated at 27 °C for 3 h. Then, methanol (50 μL/well) was added, and the plates were incubated for 10 min to fix the parasites. Absorbance was determined spectrophotometrically at 490 nm on a Tecan plate reader. DMSO was used as a negative control, and benznidazole and nifurtimox (both Sigma Aldrich, Switzerland) were used as positive controls. Results were expressed as IC_{50} and calculated by GraphPad Prism 5® from the sigmoidal concentration-response curve. All experiments were performed in triplicates at least three times.

In vitro *activity against procyclic* T. brucei brucei

T. brucei brucei in exponential growth phase were counted, adjusted to a concentration of 1.0 x 10^6 parasites/mL and exposed to plant extracts for 72 h at 27 °C. The assays were carried out in 96-well plates (100 µL/well). After 72 h of incubation, the plates were inspected under an inverted microscope to ensure the growth of the controls and sterile conditions. 10 µL MTT (5 mg/mL in PBS) were added to the plates. The cells were further incubated for 4 h at 27 °C. After incubation, the purple formazan precipitates were solubilized in DMSO (200 µL/well). Absorbance was determined spectrophotometrically at 550 nm. DMSO was used as negative, and nifurtimox as a positive control. Results were expressed as IC_{50} calculated by GraphPad Prism 5® from the sigmoidal concentration-response curve. Each assay was carried out in triplicate for the initial profiling.

Cytotoxicity measurements in HeLa, CHO and Raw 264.7 cells

Cytotoxicity/antiproliferative assays were performed by the MTT method, as reported previously (Gertsch et al., 2003). CHO-K1, HeLa, and Raw 264.7 cells were counted and adjusted to a final concentration of $2x10^4$ cells/mL. 100 µL were seeded in 96-well plates and incubated for 24 h at 37 °C with 5% CO_2. Then, 100 µL medium containing plant extracts at various concentrations (from 100 µg/mL to 0.8 µg/mL) were added. Plates were again incubated for 72 h at 37 °C with 5% CO_2. After incubation, the plates were inspected under an inverted microscope to assure the growth of the controls and sterile conditions. The medium was removed and 90 µL fresh culture medium and 10 µL MTT (5 mg/mL in PBS) were added to each well. Plates were incubated for 4 h at 37 °C with 5% CO_2. Subsequently, the purple formazan precipitate was solubilized in DMSO (200 µL/well) and absorbance determined spectrophotometrically at 550 nm. Cells cultured in the absence of compounds were used as control of viability (negative control). Results were expressed as IC_{50} or as % cell viability of negative control calculated with GraphPad Prism 5®. Each assay was carried out in triplicate in three independent experiments.

Parasite release from trypomastigote-infected cells using flow cytometry

The EtOAc extracts from the CD library and the active antitrypanosomal extracts from the *DMM* library were evaluated in a *in vitro* model of *T. cruzi* parasite release assay in CHO cells using analytical fluorescence-activated cell scanning (FACS). In this assay, it was possible to simultaneously assess inhibition of growth and release of the parasite from infected host cells, which are relevant disease parameters. CHO cells were counted and adjusted to a final concentration of $8x10^4$ cells/mL. 500 µL were seeded in 24-well plates and incubated for 24 h at 37 °C with 5% CO_2. Subsequently, cells were infected with culture-derived trypomastigotes in fresh medium without FBS at a multiplicity of infection (MOI) of 1:10 and incubated at 37 °C in 5% CO_2 for 24 h. During this time, trypomastigotes were allowed to invade host cells. After infection, the medium containing non-internalized parasites was removed and cells were washed twice with PBS. 500 µL of fresh RPMI medium without FBS containing plant extracts or controls (vehicle or benznidazole) were added. Plates were then incubated for five days at 37 °C with 5% CO_2. After incubation, the plates were inspected under an inverted microscope to assure cell viability and sterile conditions. To determine the antitrypanosomal effects, trypomastigotes (and residual amastigotes from burst cells) were collected from the extracellular medium and transferred into Eppendorf tubes. Infected CHO cells were washed twice with PBS and the washing PBS was collected in the tubes containing the released parasites. Parasites were pelleted and resuspended in PBS and incubated with 50 nM SYTO9 (S34854) dye (Thermo Scientific) for 30 min at 37 °C with 5% CO_2. Following this, the parasites were fixed

using 4% paraformaldehyde in PBS for 2 h at room temperature. Relative quantitation of the released parasite population was done via FACS on a FACScan (BD Biosciences) equipped with a solid-state laser (Cytek, Cambridge, UK) at 485 nm excitation and 535 nm emission (FL1). Each plate also contained non-infected untreated controls (blanks: 0% released parasites), infected untreated controls (negative control: 100% released parasites), and reference controls (positive control: benznidazole at 20 µM). Results were expressed as % inhibition of released parasites compared to the negative control. All experiments were performed in triplicates at least three times.

Culture-derived trypomastigotes expressing eGFP was used in the screening of hydroxylated anthraquinones. During the treatment of infected host cells with compounds until parasite release, RPMI medium supplemented with 0.5% hiFBS was used. Benznidazole was used as the positive control as mentioned above.

Reactive oxygen species detection

Intracellular levels of ROS were measured in CHO-K1 cells using 2′,7′-Dichlorofluorescin diacetate (Sigma D6883). DCFDA is a cell-permeable probe that can be de-esterified to fluorescent 2′,7′-Dichlorofluorescin intracellularly. The cells were seeded in 12-well plates in RPMI medium supplemented with 10% FBS. After 24 h, the medium was replaced with RPMI containing low FBS as performed in the parasite release assay: No FBS, 0.5% FBS and, 10% FBS as a control. The cells were washed and harvested using trypsin 24 h after changing medium and incubated with 10 µM DCFDA probe in PBS for 30 min in the dark. The fluorescence of a total of 10000 events was acquired per sample on FACS (FL-1 channel, 485 nm excitation and 535 nm emission). The fold change in DCF fluorescence was calculated in relation to cells cultured in complete medium.

Biosafety

Experimental work with live *T. cruzi* was carried out following standard operating procedures in compliance with biosafety level 3* regulations (BSL3*) approved by the safety authorities of the University and Canton of Bern, Switzerland: The Standard Operational Procedures of the experiments were reported to the Swiss Authority Federal Office of Public Health (BAG).

Comparison of EtOAc plant extract libraries

Hit rates were defined and calculated for both the Bolivian CD and the *DMM* extract libraries. For host cell cytotoxicity, the hit criterion was > 50% HeLa growth inhibition at 25 µg/mL (72 h incubation). The hit criterion for antichagasic activity was > 50% *T. cruzi* epimastigote growth inhibition at 25 µg/mL (72 h incubation). The criterion for potentially selective antichagasic activity was > 50% *T. cruzi* epimastigote growth inhibition at 25 µg/mL and < 50% inhibition of HeLa or CHO cellular proliferation at 25 µg/mL. The null hypothesis of the statistical test was that no relationship exists on the categorical variables (probability of hits) in the equally heterogeneous (genera and families) extract libraries, which were independent and with a reasonable sample size (>100 and <700). Results related to general cytotoxicity and selective antichagasic effects were compared with the X^2 test and *P*-values were calculated. A *P*-value < 0.05 was considered as statistically significant. We investigated the phylogenetic distribution of plant species with the ggtree package (36) in the R environment Version 3.5.0 (http://www.R- project.org/).

Microfractionation, bioactivity-guided isolation and structure elucidation

General Experimental Procedures. Optical rotations were measured in chloroform or methanol on a Jasco P-2000 digital polarimeter (Tokyo, Japan) with a 10 cm microcell. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer (Billerica, CA, USA) operating at 500.13 MHz for 1H and 125.77 MHz for 13C. Measurements were performed with a 1 mm TXI probe at 18 °C. Data were processed with Bruker TopSpin 3.5 software. HR-ESI-MS data were recorded in positive ion mode on a Thermo Scientific Orbitrap LQT XL mass spectrometer (Waltham, MA, USA). Centrifugal partition chromatography (CPC) was performed on an Armen Instrument (AlphaCrom, Rheinfelden, Switzerland) with coil volume 100 mL connected to a Varian pump model 210 (Agilent technologies, Santa Clara, CA, USA), a Varian 218 UV detector and a Varian fraction collector (model 704). Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) was carried out on a Puriflash 4100 system (Interchim, Montluçon, France) with a Waters (Milford, MA, USA) SunFire Prep C18 OBD column (5µm, 30 × 150mm i.d., guard column 10 × 20 mm i.d.). Semipreparative RP-HPLC was performed on an Agilent 1100 Series instrument with a DAD detector (Santa Clara, CA, USA) and a Waters (Milford, MA, USA) SunFire C18 column (5 µm, 150 × 10 mm i.d., guard column 10 × 10 mm i.d.). An HPLC system consisting of degasser, binary mixing pump, autosampler, column oven, and a diode array detector (all Shimadzu, Kyoto, Japan) connected via a T-splitter to an Alltech 3300 ELSD detector (Büchi, Flawil, Switzerland) and a Shimadzu 8030 triple quadrupole MS system with ESI and APCI interfaces was used for HPLC analysis. Data acquisition and processing were performed with LabSolution software (Kyoto, Japan). Separations were achieved with a SunFire C18 column (3.5 µm, 150 × 3.0 mm i.d., guard column 10 mm × 3.0 mm i.d.) (Waters, Milford, MA, USA). Microfractionation was carried out on the same instrument, but with a FC 204 fraction collector (Gilson, Middleton WI, USA) connected instead of the MS. Microfractions were collected into 96-deepwell plates. HPLC grade acetonitrile (Macron Fine Chemicals, Avantor Performance Materials, Phillipsburg, NJ, USA) and water from a Milli-Q water purification system (Merck, Darmstadt, Germany), and formic acid from Merck were used for HPLC separations. NMR spectra were recorded in DMSO-d6, methanol-d4, or chloroform-d (Armar Chemicals, Döttingen, Switzerland).

Microfractionation and isolation of natural products from selected extracts was carried out for subsequent testing on epimastigote viability/proliferation (72 h incubation assay) and in the parasite release assay. For microfractionation, extracts were dissolved in DMSO (10 mg/mL) and separated by gradient HPLC [0.1% aq. formic acid (A), 0.1% formic acid in acetonitrile (MeCN) (B); 0−30 min (5-100% B); flow rate 0.4 mL/min; injection volume 4 x 30 µL; room temperature]. Optimized gradients were used for *Laurus nobilis* leaf extract [0−5 min (5-30% B), 5−30 min (30-100% B)] and *Sium sisarum* root extract [0−5 min (5-40% B), 5−30 min (40-100% B)]. One-minute fractions were collected in 96-deep-well plates. Plates were dried in a Genevac EZ-2 evaporator (Ipswich, UK), and microfractions in wells redissolved in DMSO prior to bioassays. Compounds in the active time windows were isolated as follows: 200.6 mg of EtOAc extract of *Rumex crispus* rhizome were submitted to preparative RP-HPLC [water (A), MeCN (B); 0− 15 min (40−60% B), 15−30 min (60% B), 30-50 min (60-100% B); flow rate 20 mL/min; injection volume 2 × 2 mL; room temperature] afforded compounds nepodin (28.2 mg, tR 19.3 min), torachrysone (2.7 mg, tR 23.9 min), emodin (1.67 mg, tR 24.7 min), and chrysophanol (8.0 mg, tR 38.9 min). 24.0 mg of EtOAc extract of S. sisarum roots were purified by semipreparative RP-HPLC [water (A), MeCN (B); 0−5 min (5-40% B), 5−20 min (40-70% B), 20-30 min (70% B); flow rate 4 mL/min; injection volume 3 × 400 µL; room temperature] to yield falcarindiol (7.1 mg, tR 21.8 min). 30.0 mg of EtOAc extract of *Rheum rhaponticum* roots were separated by semipreparative RP-HPLC [water (A), MeCN (B); 0−20 min (5-60% B), 20−30 min (60% B); flow rate 4 mL/min; injection volume 3 × 500 µL; room temperature] to afford (6R, 7S)-costunolide (1.1 mg, tR 26.3 min). 2.0 g of *L. nobilis* leaf extract were separated by CPC [Hexane/EtOAc/MeOH/water,

7/3/5/5, v/v/v/v]; 0-60 min (descending mode), 60-72 min (ascending mode); flow rate 5 mL/min; rotation 2000 rpm; injection volume 10 mL]. Separation was monitored by the HPLC gradient mentioned above and detection at 210 nm. Active compounds were in fractions F2 (18-20 min), F6 (46-49 min), F9 (67-72 min). These fractions were further purified by semipreparative RP-HPLC [water (A), MeCN (B); flow rate 4 mL/min; injection volume 3 × 500 µL; room temperature]. F2 (32.0 mg) [0−45 min (25% B)] yielded compounds (+)-reynosin (3.8 mg, tR 26.6 min), zaluzanin C (1.6 mg, tR 29.2 min), and santamarine (2.7 mg, tR 39.7 min). F6 (13.0 mg) [0−30 min (42% B)] afforded (3S)-3-acetylzaluzanin C (4.2 mg, tR 23.8 min), and F9 (44.0 mg) [0−30 min (40-65% B)] yielded costunolide (14.1 mg, tR 21.9 min), dehydrocostus lactone (5.3 mg, tR 23.7 min), and eremanthin (2.4 mg, tR 24.7 min).

Nepodin: amorphous solid; HR-ESI-MS m/z 217.0850 $[M + H]^+$ (calc for $C_{13}H_{13}O_3^+$, 217.0859).

Torachrysone: amorphous solid; HR-ESI-MS m/z 247.0963 $[M + H]^+$ (calc for $C_{14}H_{15}O_4^+$, 247.0965).

Emodin: amorphous solid; HR-ESI-MS m/z 271.0598 $[M + H]^+$ (calc for $C_{15}H_{11}O_5^+$, 271.0601).

Chrysophanol: amorphous solid; HR-ESI-MS m/z 255.0649 $[M + H]^+$ (calc for $C_{15}H_{11}O_4^+$, 255.0652).

(3R, 8S)-Falcarindiol: oil; $[\alpha]_D^{25}$ +285 (0.1, $CHCl_3$); APCI-MS m/z 262 $[M + H]^+$.

(6R, 7S)-Costunolide: amorphous solid; $[\alpha]_D^{25}$ +108 (0.1, $CHCl_3$); HR-ESI-MS m/z 233.1527 $[M + H]^+$ (calc for $C_{15}H_{21}O_2^+$, 233.1536).

(+)-Reynosin: amorphous solid; $[\alpha]_D^{25}$ +59 (0.2, CH_3OH); ESI-MS m/z 249 $[M + H]^+$.

Zaluzanin C: amorphous solid; $[\alpha]_D^{25}$ +37 (0.1, $CHCl_3$); ESI-MS m/z 247 $[M + H]^+$.

Santamarine: amorphous solid; $[\alpha]_D^{25}$ +83 (0.1, CH_3OH); ESI-MS m/z 249 $[M + H]^+$.

(3S)-3-acetylzaluzanin C: amorphous solid; $[\alpha]_D^{25}$ +15 (0.1, $CHCl_3$); ESI-MS m/z 289 $[M + H]^+$.

Dehydrocostus lactone: amorphous solid; $[\alpha]_D^{25}$ -6 (0.1, $CHCl_3$); ESI-MS m/z 231 $[M + H]^+$.

Eremanthin: amorphous solid; $[\alpha]_D^{25}$ -276 (0.1, $CHCl_3$); ESI-MS m/z 231 $[M + H]^+$.

For the SAR analysis with 9,10-anthracenedione, the following commercial compounds were purchased (≥95% purity). Anthraquinone (**1**), alizarin (**4**), alizarin RedS (**5**), quinizarin (**6**), anthrarufin (**7**), anthraflavic acid (**9**), emodin (**2**), purpurin (**3**), rhein (**15**), diacerein (**16**), chrysophanol (**10**), disperse Red11 (**17**) and dantron (**8**) were all obtained from Sigma Aldrich, Switzerland. Aloe emodin (**11**), physcion (**12**), 2-hydroxy-3-methylanthraquinone (**13**) and 2-hydroxy-1-methylanthraquinone (**14**) were obtained from Toronto Research Chemical, ON, Canada. Aurantio-obtusin (**18**) was obtained from AdooQ Biosciences LLC, CA, USA.

Supplementary Figures

Figure S1. Selected Bolivian plant species used to treat symptoms related to CD. A. *Senna chloroclada* flowers and roots were the most frequently cited botanical drugs among the Izoceño-Guaraní and showed significant selective antichagasic effects on cellular parasite release *in vitro*. **B.** *Acanthostyles buniifolius* was used by the Quechua and exhibited selective antichagasic activity *in vitro*. **C.** Chiquitano informant cutting tree bark of *Jacaranda cuspidifolia.* **D.** The roots of *Galphimia brasiliensis* are used by the Chiquitano to treat symptoms related to CD. Related to Figure 1.

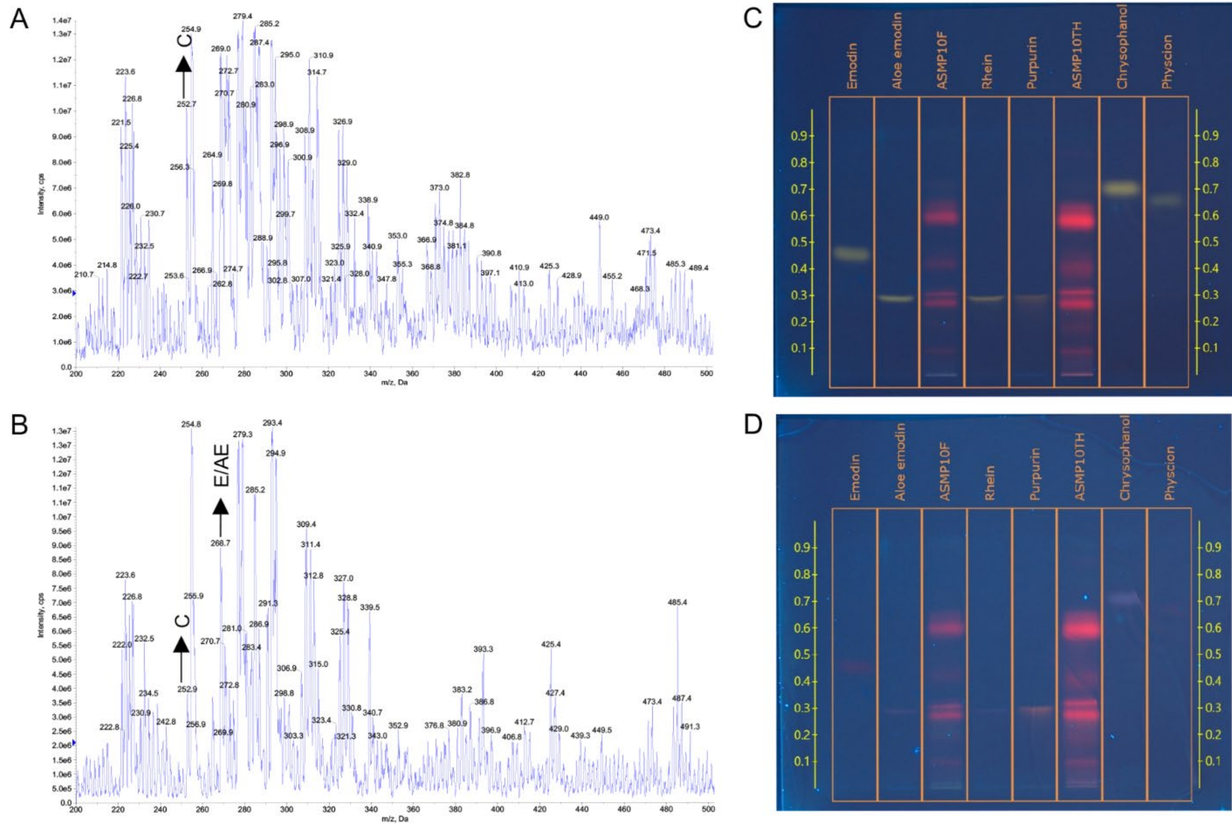

Figure S2. Preliminary analysis of *S. chloroclada* extracts by full scan Q1 in negative mode and thin-layer chromatography (TLC) showing the presence of anthraquinones. A. MS spectra of ethyl acetate extracts of *S. chloroclada* A. flower (10F) and **B.** areal parts (10TH) used in the *in vitro* assays were infused at 5 µg/mL in 90% acetonitrile. The scans were made on a AB Sciex 5500 Q-Trap device. **C.** TLC chromatograms of extracts were developed in petrol ether: ethyl acetate: formic acid (75:25:1, v/v/v) followed by **D.** spraying with 10% methanolic potassium hydroxide. The samples were sprayed using CAMAG® Automatic TLC sampler ATS4. TLC plate was visualized under UV 366 nm. E: emodin, C: chrysophanol, AE: aloe-emodin. Related to Figure 2.

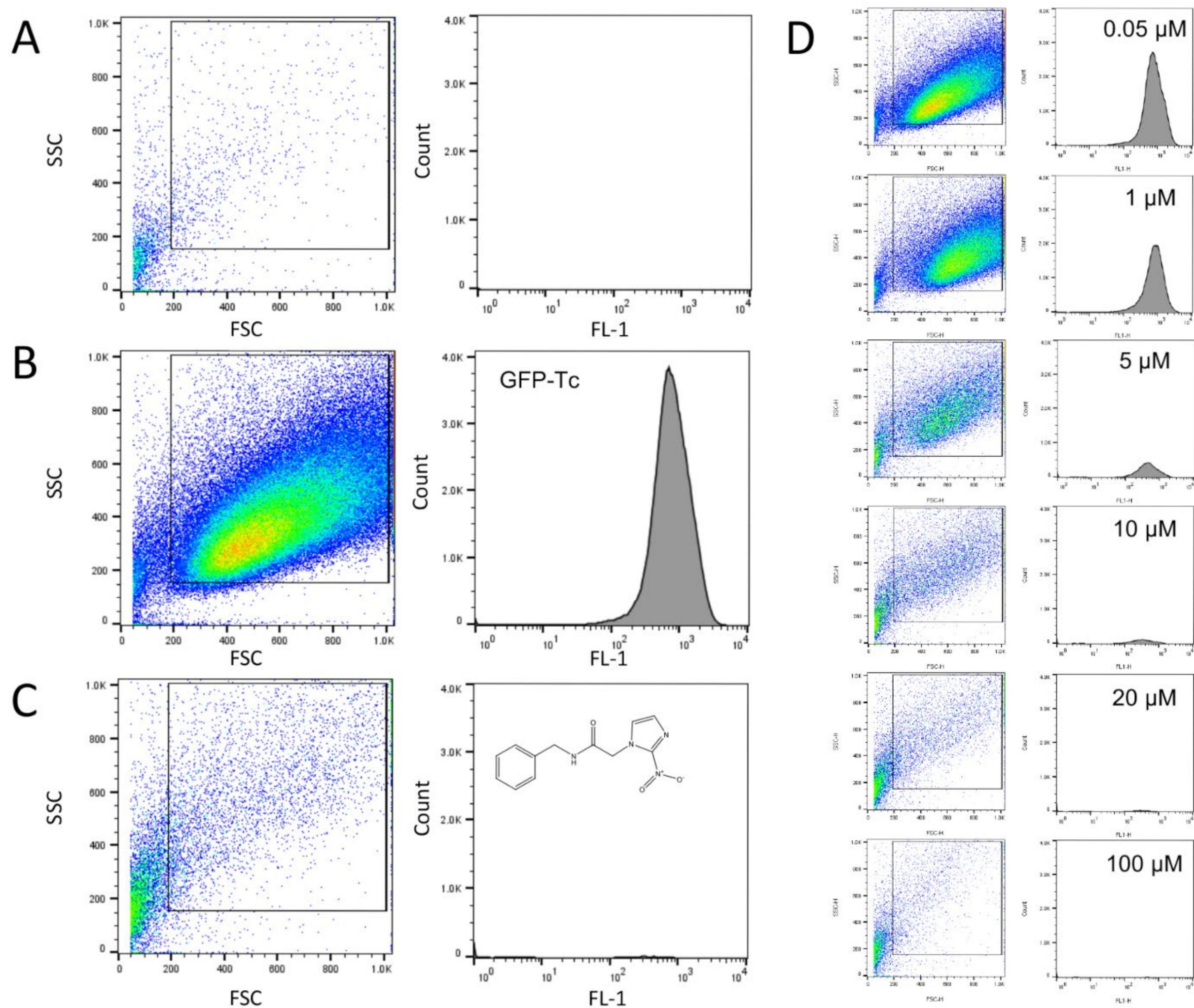

Figure S3. **FACS-based parasite release assay in *T. cruzi* infected CHO cells.** CHO cells were infected with GFP-expressing trypomastigotes (10:1) for 24 h. The cells were then washed and fresh medium supplemented with 0.5% hiFBS was added. Parasites released into the media at 6dpi were fixed with 4% paraformaldehyde, and collected for FACS analysis. **A.** Negative control of non-infected cells at 6 dpi showing FSC/SSC and FL-1 histograms. **B.** Positive control showing fluorescence associated to GFP expression in parasites at 6 dpi, showing FSC/SSC and FL-1 histograms. **C.** Representative example of benznidazole (BZN) treatment (20 µM) at 6 dpi, showing FSC/SSC and FL1 histograms illustrative of reduced parasite release. **D.** Illustration of the concentration-dependent effect of benznidazole leading to decreased release of parasites in the medium at 6 dpi with a calculated IC_{50} value of 1.35 µM (95% CI 1.05-1.63 µM). Related to Figure 6.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
SYTO9	Thermo Fisher Scientific	S34854
2′,7′-Dichlorofluorescin diacetate	Sigma	D6883
Methylthiazolyldiphenyl-tetrazoliumbromid	Sigma	298-93-1
2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide	Thermo Fisher Scientific	X6493
Experimental Models: Cell Lines		
Trypanosoma cruzi (ATCC 50832)	American Type Culture Collection	ATCC 50832
CHO-K1	American Type Culture Collection	ATCC CCL-61
RAW 264.7	American Type Culture Collection	ATCC TIB-71
HeLa	American Type Culture Collection	ATCC CCL-2
Software and Algorithms		
FlowJo	Becton Dickinson	v 10
Prism	GraphPad	v 8