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REMARKABLE ANTI-*TRYPANOSOMA CRUZI* ACTIVITY IN SELECTED SALVADORAN PLANT SPECIES

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ABSTRACT: In Latin America, Chagas is one of the most prevalent diseases, and current chemotherapy with benznidazole and nifurtimox is ineffective in chronic phase. Therefore, the development of new drugs, especially for the chronic stage of the infection, is urgently needed. Performing an *in-vitro* anti-trypanosome assay, we analyzed 114 plant species from Salvadoran flora, resulting in 34 active plants against epimastigote of *Trypanosoma cruzi* belonging mainly to the botanical families Piperaceae, Asteraceae, Salicaceae, Annonaceae, and Acanthaceae. Thus, nine of them showed prominent activity between 91.18 to 98.94% of viability suppression at 100 µg/mL (*Annona holosericea*, *Calea urticifolia*, *Eremosis leiocarpa, Peperomia pseudoalpina*, *Piper amalago, Piper martensianum*, *Casearia corymbosa*, *Piparea dentata* and *Solanum nudum*), highlighting *C. corymbosa*, *P. pseudoalpina* and *P. martensianum* as the most promising species as sources of natural compounds useful for the treatment of Chagas disease. *P. speudoalpina* and *P. martensianum* do not have reports of their phytochemical composition or biological activities.

INTRODUCTION: Chagas disease is caused by *Trypanosoma cruzi* protozoan and affects around 6-7 million people in Latin America causing around 50,000 deaths per year. On the other hand, 65-100 million people are living in infection risk [1]. A study on Chagas disease in El Salvador has provided important epidemiological information between the years 2018 through 2020, including a 34.4% infection rate among triatomines, vectors of Chagas disease, and a 2.3% seropositivity rate for pediatric Chagas in Sonsonate. In 2022, a maternal surveillance study showed a 6% *T. cruzi* positivity rate among pregnant women [2]. During that year, the Ministry of Public Health reported 52 acute and 894 chronic suspected Chagas cases [3].

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Plant-derived natural products are essential in drug discovery and development, providing a rich source of bioactive molecules with diverse properties. In fact, a quarter of currently useful drugs are derived from medicinal plants. This is especially important in regions where trypanosomiases are prevalent [4].

Countries like El Salvador, which have abundant medicinal plant resources, are of particular interest. Some of these plants and their compounds have demonstrated efficacy *in-vitro* against *T. cruzi* [5, 6]. Herein, we report the *in-vitro* activity of 114 plant species from 14 botanical families against *T. cruzi* epimastigotes. Notably, nearly 30% of the evaluated species exhibited antitrypanosomal activity.

MATERIALS AND METHODS:

Plant Selection: The species were selected based on the results of a previous investigation [5], in which certain families and botanical genera existing in the Salvadoran flora were promising. 114 plant species were collected in different areas of El Salvador in 2019-2022 and identified by Jenny Elizabeth Menjívar Cruz from the Museo de Historia Natural de El Salvador and Gabriel Cerén from the Escuela de Biología, Universidad de El Salvador. A voucher specimen has been deposited for each species in the Herbarium at the MHES **Table 1**.

Preparation of Plant Extracts: The listed plants in **Table 1** were ground to a particle size of less than 2 mm (Bel-Art products, USA, model micro-mill) after drying at 40 °C for 48 to 72 hours in an air-circulating oven (BIOBASE, China, model BOV-V225F). An ultrasonic bath with magnetic stirrer (VWR, USA, model 97,043-988, operating frequency 35 kHz) was used to extract 20 grams of each sample over the course of 90 minutes at 25 °C. Each extract was concentrated under reduced pressure at 40 °C (model RE801, Yamato Scientific Co., Ltd., Japan) to create the crude MeOH extracts.

TABLE 1: ANTITRYPANOSOMAL ACTIVITY OF SALVADORAN SPECIES AGAINST EPIMASTIGOTE STAGE OF T*RYPANOSOMA CRUZI*

Family/Scientific name	Plant part used	Voucher/Place	Viability (%)	
			100 µg/mL	10 µg/mL
Acanthaceae	Aerial parts			
Aphelandra scabra (Vahl) Sm.		GC-4819/1	48.19±3.78	72.46±0.93
Ruellia inundata Kunth	Aerial parts	GC-4816/1	49.60±2.40	78.62±5.89
Achatocarpaceae	Aerial parts			
Achatocarpus nigricans Triana		GC-4811/1	62.67±3.98	72.28±7.47
Anacardiaceae	Leaves			
Schinus terebinthifolia Raddi		GC-5657/2	75.75±1.26	77.42±8.12
Annonaceae	Leaves	GC-5233/3	46.07±2.49	75.09±1.69
Annona cherimola Mill.	Stem bark	GC-5233/3	69.50±10.22	93.30±5.02
Annona holosericea Saff.	Stem bark	GC-5326/4	8.82±0.52	89.45±10.25
Annona reticulata L.	Stem bark	GC-519/5	88.91±1.27	100.29±8.56
Sapranthus microcarpus (Donn.Sm.) R.E.Fr.	Leaves	GC-5029/6	58.43±2.45	81.88±8.33
	Aerial parts	GC-5272/8	57.31±11.39	85.86±10.29
Sapranthus violaceus (Dunal) Saff.	Leaves	GC-5148/4	68.09±11.51	80.89±2.61
	Stem bark	GC-5148/4	55.70±10.14	80.38±7.85
Apocynaceae				
Alstonia longifolia (A.DC.) Pichon	Aerial parts	GC-4823/1	34.75±0.72	78.07±1.45
Echites yucatanensis Millsp. ex Standl.	Leaves	GC-4888/7	60.36±5.94	76.61±6.80
Ruehssia veronicae (W.D.Stevens) L.O. Alvarado	Aerial parts	GC-5341/4	78.11±8.11	91.14±5.27
Aristolochiaceae				
Aristolochia anguicida Jacq.	Aerial parts	JM-5121/9	50.90±9.72	96.05±4.98
Aristolochia grandiflora Sw.	Aerial parts	GC-5190/5	67.50±4.35	82.78±4.49
Aristolochia maxima Jacq.	Aerial parts	JM-5122/9	80.53±8.30	104.13±4.89
Aristolochia stevensii Barringer	Aerial parts	JM-5128/10	81.88±8.74	106.35±1.42
Asteraceae				
Ageratum corymbosum Zuccagni	Aerial parts	JM-4634/11	47.46±5.32	94.02±1.84
Calea urticifolia DC.	Leaves	GC-5656/2	2.17±0.65	56.17±8.3
Chromolae naodorata (L.) R.M.King & H.Rob.	Aerial parts	JM-5216/12	59.96±13.36	72.09±1.67
Eremosis leiocarpa (DC.) Gleason	Aerial parts	GC-5150/4	8.82±1.71	75.39±6.20
Fleischmannia hymenophylla (Klatt) R.M.King & H.Rob.	Aerial parts	JM-4647/10	80.03±3.16	99.91±1.61
Fleischmannia pratensis (Klatt) R.M.King & H.Rob.	Aerial parts	JM-4646/10	93.79±0.90	97.34±6.24
Fleischmann iopsis leucocephala (Benth.) R.M.King &H.Rob	Aerial parts	GC-4885/7	50.83±5.79	75.30±5.03

Lepidaploa canescens (Kunth) H.Rob.	Aerial parts	JM- 5217/12	65.17±3.50	75.13±3.85
Perymenium nicaraguense S.F. Blake	Leaves	JM-5149/12	73.37±4.22	101.94±2.04
Salmea scandens DC.	Aerial parts	GC-4857/13	45.67±1.82	80.78±9.89
Schistoca rphahondurensis Standl. & L.O. Williams	Aerial parts	GC-4856/13	50.79±8.75	73.50±7.31
Tagetes filifolia Lag.	Whole plant	GC-4710/12	92.13±8.61	102.61±10.23
Tagetes tenuifolia Cav.	Aerial parts	JM-5205/11	67.95±11.21	92.13±3.59
Verbesina turbacensis Kunth	Aerial parts	JM-5218/12	67.96±8.98	92.33±4.76
Vernonanthura patens (Kunth) H.Rob.	Aerial parts	GC-5152/4	74.78±3.26	85.56±5.95
Bixaceae				
Cochlospermum vitifolium (Willd.) Spreng.	Stem bark	GC-5273/8	74.95±7.44	88.32±8.55
Boraginaceae				
Cordia panamensis L. Riley	Leaves	GC-5030/6	58.58±9.90	93.33±8.42
	Stem bark	GC-5325/4	77.15±7.41	90.10±4.64
Burseraceae				
Bursera bipinnata Engl.	Stem bark	GC-5317/4	59.05±12.55	108.08±2.60
Campanulaceae				
Lobelia laxiflora Kunth	Aerial parts	GC-5134/4	60.25±8.01	87.09±1.52
Capparaceae				
Crateva tapia L.	Leaves	GC-4830/1	50.37±1.01	80.86±6.35
Convolvulaceae				
Ipomoea alba L.	Aerial parts	GC-5031/6	82.26±7.03	89.13±3.41
Ipomea wolcottiana Rose	Leaves	GC-5274/8	49.10±2.83	84.38±12.88
	Stem bark	GC-5274/8	92.66±7.33	98.17±6.36
Ipomoea aurantiaca L.O. Williams	Aerial parts	GC-5309/4	76.09±7.08	84.59±1.48
Ericaceae				
Gaultheria erecta Vent.	Leaves	JM-5201/11	75.60±3.73	97.47±5.91
Erythroxilaceae				
Erythroxylum mexicanum Kunth	Leaves	GC-4976/8	55.09±6.10	85.70±4.88
Euphorbiaceae				
Acalypha firmula Müll. Arg	Aerial parts	JM-5148/12	59.78±3.07	87.03±1.68
Croton pseudo niveus Lundell	Leaves	GC-4979/8	77.37±9.00	98.49±5.44
Croton repens Schltdl.	Aerial parts	GC-4682/12	69.99±3.83	96.90±4.09
Omphalea oleifera Hemsl	Stem bark	GC-5281/8	77.42±0.96	93.00±2.28
Fabaceae				
Crotalaria sagittalis L.	Aerial parts	GC-4984/6	73.00±7.34	101.95±1.73
Crotalaria vitellina Ker Gawl.	Aerial parts	GC-4995/6	61.06±2.82	86.28±13.13
Desmodiumni caraguense Oerst.	Aerial parts	GC-4815/1	58.61±14.75	84.64±12.44
Erythrina lanceolata Standl.	Aerial parts	GC-5328/4	76.60±1.56	91.27±3.44
Leptolobium panamense (Benth.) Sch. Rodr. & A.M.G.Azevedo	Leaves	GC-5158/4	67.73±10.85	82.17±11.92
Lonchocarpus phaseolifolius Benth.	Stem bark	GC-5278/8	87.45±5.30	89.32±12.09
*Machaeriumbio vulatum*Micheli	Leaves	GC-4968/8	78.03±4.12	84.02±8.41
Machaerium kegelii Meisn.	Leaves	GC-4905/7	67.43±4.18	81.44±0.97
Machaerium pittieri J.F.Macbr.	Aerial parts	GC-4782/4	48.09±5.86	72.35±4.10
	Leaves	GC-4899/7	71.09±5.33	88.16±8.12
Mimosa diplotricha C.Wright	Aerial parts	JM-5146/12	76.21±4.36	98.85±9.79
Mimosa pudica L.	Aerial parts	JM-5136/14	65.19±2.55	88.52±3.31
Pachyrhizus erosus (L.) Urb.	Aerial parts	GC-4678/12	78.25±12.78	91.01±4.89
Poiretia punctata (Willd.) Desv.	Aerial parts	GC-5021/6	67.86±2.89	81.85±2.21
Rhynchosia phaseoloides (Sw.) DC.	Leaves	GC-5199/15	76.34±12.96	100.73±6.01
Fagaceae *Quercus skinneri* Benth.	Leaves	GC-5261/16	58.75±11.19	85.17±4.92
Gesneriaceae				
Drymonia serrulata Mart.	Aerial parts	GC-5024/6	75.03±8.04	87.40±2.57
Lamiaceae				
Hyptis capitata Jacq.	Aerial parts	GC-4788/4	61.47±7.42	79.34±12.96
Salvia urica Epling	Aerial parts	GC-4676/12	64.82±11.28	81.70±2.33
Lauraceae				
Damburneya martinicensis (Mez) Trofimov	Branch	JM-5125/10	66.33±11.68	92.57±2.86
Ocoteasinuata (Mez) Rohwer	Leaves	JM-5131/10	59.95±11.78	108.74±5.77
Malvaceae				

Helicteresgu azumifolia Kunth	Aerial parts	GC-5020/6	57.89±7.49	80.72±10.90
Meliaceae				
Trichilia martiana C.DC.	Fruit	GC-4892/7	75.33±8.96	85.04±2.67
Trichilia trifolia L.	Leaves	JM-5116/9	40.05±13.56	85.14±12.67
Myricaceae				
Myrica cerifera L.	Leaves	JM-5197/11	61.65±13.19	78.23±12.09
Myrtaceae				
Eugenia cacuminum Standl. & Steyerm.	Leaves	GC-4789/4	28.06±12.49	82.25±2.39
Onagraceae		J		
Fuchsia microphylla subsp. *chiapensis* (Brandegee)	Leaves	M-5199/11	58.63±11.95	91.89±3.12
P.E.Berry & Breedlove	Branch	JM-5199/11	67.40±9.00	100.71±7.36
Phytolaccaeae				
Phytolacca rugosa A.Braun & C.D. Bouché	Aerial parts	GC-4859/13	62.94±3.22	87.11±8.12
Piperaceae				
Peperomia bernouillii C.DC.	Whole plant	GC-5182/5	39.15±6.72	74.95±9.20
Peperomia galioides Kunth	Aerial parts	GC-4842/13	49.09±6.68	82.89±9.89
Peperomia lanceolatopeltata C.DC.	Whole plant	JM-5137/14	46.04±10.81	85.92±9.93
*Peperomia lancifolia*Hook.	Whole plant	GC-5186/5	42.79±5.00	73.61±1.93
*Peperomia lignescens*C.DC	Whole plant	GC-4920/17	48.86±0.31	90.76±3.46
*Peperomia pseudoalpina*Trel.	Whole plant	GC-5105/4	1.25±0.35	68.18±7.78
Peperomia sanfelipensis C.DC. ex Donn.Sm.	Aerial parts	GC-4845/13	44.68±4.68	88.68±0.86
Piper aduncum L.	Aerial parts	GC-4784/4	30.88±12.50	76.03±4.31
Piper amalago L	Leaves	GC-4814/1	3.22±1.20	66.24±8.73
*Piper glabrescens*C.DC.	Aerial parts	GC-4790/4	37.56±7.35	72.56±3.79
Piper martensianum C.DC.	Aerial parts	GC-4785/4	1.62±0.50	70.22±7.36
*Piper marginatum*Jacq.	Aerial parts	GC-5184/5	57.08±3.78	76.67±5.04
*Piper tuberculatum*Jacq.	Leaves	GC-4963/8	17.79±11.17	83.46±5.06
Piper yucatanense C. DC.	Aerial parts	GC-4962/8	25.48±5.57	71.97±11.69
Podocarpaceae				
Podocarpus oleifolius D.Don	Leaves	JM-5129/10	48.27±11.31	95.27±15.35
Polygalaceae				
Asemeia violacea (Aubl.) J.F.B.Pastore & J.R.Abbott	Aerial parts	GC-4680/12	62.35±8.69	98.13±11.22
Polygala paniculata L.	Aerial parts	GC-5654/12	51.42±12.67	88.83±9.17
Proteaceae				
Roupala Montana Aubl.	Leaves	JM-5203/11	81.57±5.57	118.36±13.42
Rhamnaceae				
Karwinskia calderonii Standl.	Leaves	GC-4778/4	60.30±3.65	81.73±5.26
Rubiaceae				
Arachnothryx laniflora (Benth.) Planch.	Aerial parts	GC-4853/13	54.68±7.42	72.94±4.02
Calycophyllum candidissimum (Vahl) DC.	Stem bark	GC-5280/8	79.67±3.38	71.89±2.69
Coccocypselum hirsutum Bartl. ex DC.	Fruit	GC-5655/12	110.89±3.81	104.42±6.63
Donnellyanthus deamii (Donn.Sm.) Borhidi	Leaves	GC-5028/6	71.19±13.24	77.01±7.47
Geophila repens (L.) I. M. Johnst.	Whole plant	GC-4966/8	66.77±6.10	86.19±9.58
Palicourea padifolia (Willd. ex Schult.) C.M.Taylor & Lorence	Root	GC-5259/13	95.81±2.89	102.32±4.73
Palicourea pubescens (Sw.) Borhidi	Aerial parts	GC-5225/15	64.33±11.29	98.69±2.41
Randia monantha Benth.	Leaves	GC-4828/1	18.79±7.37	77.73±11.37
Rovaeanthus strigosus (Benth.) Borhidi	Aerial parts	JM-5200/11	90.55±2.05	101.99±3.18
Solenandra mexicana (A.Gray) Borhidi	Aerial parts	GC-5269/8	75.09±6.62	79.20±8.23
Rutaceae				
Casimiroa edulis La Llave	Leaves	GC-5198/15	57.03±11.41	99.63±6.42
	Stem bark	GC-5198/15	59.47±1.81	90.93±2.02
Pilocarpus racemosus Vahl	Leaves	GC-5235/3	47.84±6.63	85.21±2.73
	Stem bark	GC-5235/3	83.14±11.24	91.66±2.49
Zanthoxylum fagarasubsp. aguilarii (Standl. & Steyerm.) Reynel	Aerial parts	GC-4796/4	41.57±3.84	74.23±6.62
Salicaceae				
Casearia corymbosa Kunth	Stembark	GC-5170/9	29.39±6.91	79.24±9.74
	Leaves	GC-5222/15	1.12±0.12	82.70±12.32
Pipar eadentata Aubl.	Aerial parts	GC-5239	1.06±0.22	77.96±7.46

Prockia crucis P. Browne ex L.	Aerial parts	GC-5171/4	67.80±5.96	78.54±3.75
Xylosma flexuosa (Kunth) Hemsl.	Leaves	GC-4900/7	40.29±11.84	74.52±8.26
	Aerial parts	GC-5231/3	86.68±3.26	97.64±11.39
Xylosma velutina (Tul.) Triana &Planch.	Aerial parts	GC-5318/4	61.39±11.75	75.68±8.20
Sapindaceae				
Paullinia cururu L.	Aerial parts	GC-5320/4	52.14±4.02	78.64±10.70
Solanaceae				
Browallia americana L.	Aerial parts	GC-5133/4	73.38±5.64	83.14±4.41
Lycianthes arrazolensis (Coult. & Donn.Sm.) Bitter	Leaves	GC-5337/4	72.95±10.72	74.59±5.19
Solanum nudum Dunal	Leaves	GC-4969/8	56.63±5.80	71.29±2.25
	Fruit	GC-4969/8	8.36±4.80	88.76±1.33
Viburnaceae				
Viburnum hartwegii Benth.	Leaves	JM-5196/11	68.53±13.61	94.51±5.84
	Stem bark	JM-5196/11	83.43±7.06	103.83±1.17

Scientific names according to The World Flora Online (July 7th, 2024): http://www.worldfloraonline.org. **Place**: Coatepeque Lake, El Congo, Santa Ana (1); University of El Salvador, San Salvador (2); Cantón Zapúa, Jujutla, Ahuachapán (3); Cantón El Limo, Metapán, Santa Ana (4); Cantón Pushtán, Nahuizalco, Sonsonate (5); Guazapa Hill, Guazapa, San Salvador (6); Las Termopilas Farm, Chiltiupán, La Libertad (7); San Jorge Farm, San Julián, Sonsonate (8); PNA San Diego La Barra, Metapán, Santa Ana (9); NP Montecristo, Metapán, Santa Ana (10); Volcano NP, Santa Ana (11); EP El Manzano, Dulce Nombre de María, Chalatenango (12); Verde Lagoon, Apaneca, Ahuachapán (13); Cantón El Amatillo, Las Vueltas, Chalatenango (14); El Pacayal Volcano, Chinameca, San Miguel (15); Las Ninfas Lagoon, Apaneca, Ahuachapán (16) and Chichicastepec Hill, Apaneca, Ahuachapán (17); PNA: Protected Natural Area; NP: National Park and EP: Ecological Park. The viabilities are represented by means of three replicates ± standard deviations (SD).

Antitrypanosomal Screenig of Crude Extracts: The antitrypanosome luminescence assay was performed as described by Castillo *et al.* 2022 [5], with some modifications. In white 384-well plates (Optiplate, Perkin Elmer) 50 µL of *Trypanosoma cruzi* epimastigotes (strain CL-Brener) at a concentration of 9x105 suspended in GIT medium supplemented with hemin (5 mg/500 mL) and 10% inactivated fetal bovine serum were placed. These were incubated at 28°C for 24 hours after adding 0.5 µL of plant extracts at 10 and 1 mg/mL concentrations in DMSO. Each extract was evaluated in triplicate. Benznidazole and Tamoxifen (10 mM, 5 mM, 1.25 mM, 0.25 mM) were used as positive control and GIT medium without epimastigotes, GIT medium with parasites plus DMSO and GIT medium with DMSO were used as blanks. After incubation, 20 µl of Cell Titer-Glo ® 2.0 reagent (PROMEGA) was added to each well and incubated for 15 minutes at 28°C protected from light. Luminescence was measured on a Biotek Synergy HTX multimode plate reader at 28°C. The results are expressed in viability percent see **Table 1**.

RESULTS: The antitrypanosomal screening was carried out with the resulting 127 methanolic dried extracts from 114 plant species collected **Table 1**.

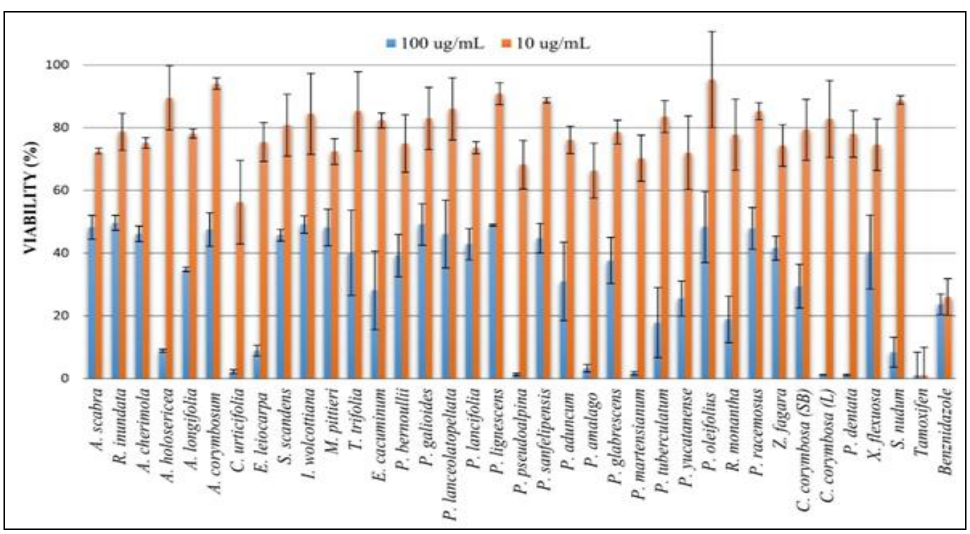

FIG. 1: ANTITRYPANOSOMAL ACTIVITY OF SALVADORAN ACTIVE SPECIES AGAINST EPIMASTIGOTE STAGE OF *TRYPANOSOMA CRUZI*

The yields from aerial part extracts ranged from 4.6-17.9%, whole plant 6.2-20.7%, stem bark 4.3-22.1%, branches 2.3-4.8%, fruits 10.4-16.9%, and roots 3.5%. The activity of antitrypanosomal screening is expressed in viability percent and summarized in **Table 1**. Considering previous criteria [5], 34 species from 14 botanic families were considered as active (less than 50% of viability percent at 100 µg/mL) **Fig. 1**. The botanic families Piperaceae, Asteraceae, Salicaceae, Annonaceae, and Acanthaceae, exhibited the highest number of active botanical species, **Fig. 2**. Among these, the aerial parts (43%), leaves (34%), and whole plant (14%) were the organs and parts of the active plants that showed activity, followed by stem bark (6%) and fruits (3%) **Table 1**.

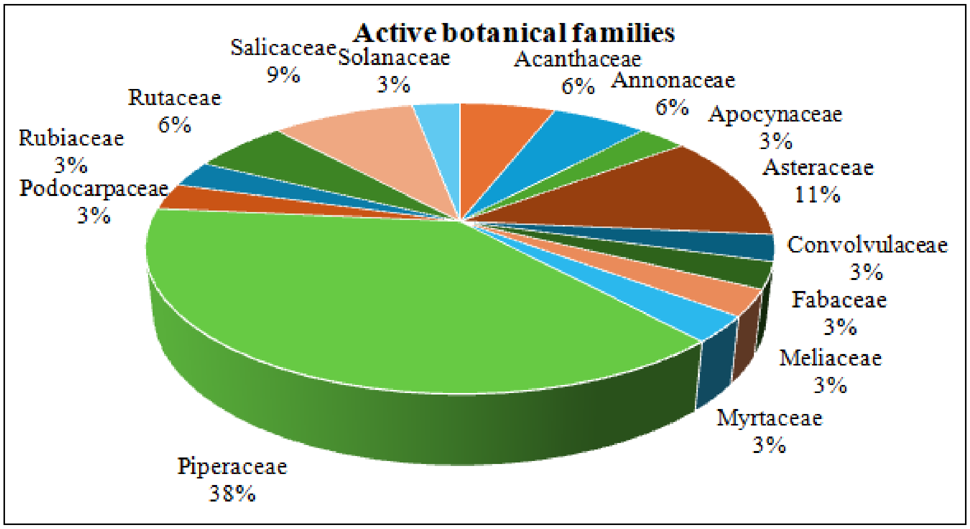

FIG. 2: ACTIVE SALVADORAN BOTANICAL FAMILIES AGAINST EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* (VIALIBILITY < 50%)

DISCUSSION: From the 34 active species identified, only 9 demonstrated remarkable activity against *T. cruzi* epimastigote, showing more than 90% suppression of epimastigote viability at a concentration of 100 µg/mL **Fig. 1** and **Table 2.** Among these, *Annona holosericea* (Annonaceae) exhibited a viability suppression of 91.18±0.52% at 100 µg/mL, **Table 2.** Annonaceae family and specifically the genus *Annona* is known to have a broad spectrum of biological activities, including antineoplastic, antiparasitic, cytotoxic, antitumoral, antiprotozoal, antidiabetic, anti-inflammatory, hepatoprotective, and analgesic properties [7, 8, 9]. There are no studies specifically addressing the antitrypanosomal activity of *A. holosericea*. However, *A. crassiflorais* traditionally used in the treatment of Chagas disease [10, 11]. Moreover, there are some reports that *A. foetida*, *A. crassiflora*, *A. muricata*, *A. squamosa*, *A. haematantha*, *A. purpurea*, *A. coriacea*, and *A. senegalensis* are active against *T. cruzi* [12, 7, 4] and some studies suggest that antitrypanosomal activity is attributed to alkaloids, acetogenins, and terpenes [10, 11, 12, 7].

TABLE 2: SALVADORAN SPECIES WITH PROMINENT ANTITRYPANOSOMAL ACTIVITY AGAINST EPIMASTIGOTE STAGE OF *TRYPANOSOMA CRUZI*

Family/Scientific name	Vernacular name	Plant part used	Viability suppression (%) at 100 µg/mL[2]
Annonaceae			
Annona holosericea Saff.	"Sincuyita"	Stem bark	91.18±0.52
Asteraceae			
Calea urticifolia DC.	"Juanislama"	Leaves	97.83±0.65
Eremosis leiocarpa (DC.) Gleason	"Palo del asma"	Aerial parts	91.18±1.71
Piperaceae			
Peperomia pseudoalpina Trel.	"Hoja de tres piedras"	Whole plant	98.75±0.35
Piper amalago L	"Santa María"	Leaves	96.78±1.20
Piper martensianum C. DC.	"Candelillo"	Aerial parts	98.38±0.50
Salicaceae			

Casearia corymbosa Kunth	"Canjurillo"	Leaves	98.88±0.12
Piparea dentata Aubl.	"Camarón rojo"	Aerial parts	98.94±0.22
Solanaceae			
Solanum nudum Dunal	"Palo del golpe"	Fruit	91.64±4.80

Scientific names according to The World Flora Online:http://www.worldfloraonline.org (July 7th, 2024). The viability suppressions are represented by means of three replicates ± standard deviations (SD).

Among the Asteraceae family exist many subfamilies, genera, and species, herbs and shrubs being the most common; this is a representative family for trypanocidal activity [13, 14 15]. In which alkaloids, flavonoids, phenolic acids, coumarins, terpenoids, quinolines and diterpenoids, triterpenoid sesquiterpene lactones, and pyrethrins are associated with antiparasitic activity [15].

In our results, *Calea urticifolia* showed an important activity (viability suppression at 100 µg/mL: 97.83±0.65%) against epimastigotes, **Table 2**; this specie is commonly known in El Salvador as "Juanislama" and used to treat gastric ulcers and as a bactericide [16]. Some studies of *C. urticifolia* have resulted in the isolation of sesquiterpene lactones (germacranolides and heliangolides), some phenols, as well as isoeugenol and phloroglucinol derivatives [17, 18, 19, 14].

This is the first report of antitrypanosomal activity for *C. urticifolia*,and it may be attributed to the presence of sesquiterpene lactones and phenolic compounds [15]. On the other hand, *C. uniflora* has demonstrated *in-vitro* activity against trypomastigote form of *T. cruzi* [20, 21, 22].

Regarding *Eremosis leiocarpa* is used in El Salvador to treat asthma [23], and was known as *Vernonia leiocarpa* (viability suppression at 100 µg/mL: 91.18±1.71%) **Table 2**, this specie does not have any reports of antitrypanosome activity, but, some sesquiterpene lactones (glaucolide F, G and H) were detected in *E. leiocarpa* [24] that could be antitrypanosomal promising compounds. Furthermore, species of this genera, including *V. auriculifera, V. brasiliana, V. guineensis, V. subuligera, V. polyanthes, V. scorpioides* have been identified as a source of trypanocidal compounds [23, 25].

Among the species of the Piperaceae family that showed greater antitrypanosomal activity, *Peperomia pseudoalpina* (viability suppression at 100 µg/mL: 98.75±0.35%), *Piper amalago* (viability suppression at 100 µg/mL: 96.78±1.20%), and *P. martensianum* (viability suppression at 100 µg/mL: 98.38±0.50%) were found, **Table 2**. This botanic family is used in traditional medicine to treat several ailments and protozoal disease [26, 27, 5], moreover, they are known for being a source of secondary metabolites with antitrypanosomal, antileishmanial, anxiolytic, anticonvulsant and antiinflammatory activities [28].

Despite its biological importance and its prominent antitrypanosomal activity found out, *Peperomia pseudoalpina* is underexplored. Likewise, no report so far has been found in the literature on the phytochemical and biological screening also, some species of *Peperomia* genera have been reported with this activity [5, 6]. Additionally, some bioactive compounds were reported with trypanocide activity against epimastigote and trypomastigote of *T. cruzi*, such as tetrahydrofuran lignans [29, 30, 6].

Tetrahydrofuran lignans being the presumed responsibles for the activity in *Peperomia* in the same way as phenylpropanoids. *P. amalago* has some pharmacological properties such as anti-inflammatory, antimicrobial, cicatrizing, antioxidant, and antileishmanial [31, 32]. Its phytochemical roots composition is mainly integrated by sesquiterpenes, pyrrolidines, and isobutylamides [31]. Two pyrrolidine alkaloids (N-[7-(3', 4'-methylenedioxyphenyl) - 2(*E*), 4(*E*)-heptadienoyl] pyrrolidine and N-[7-(3', 4'-methylenedioxyphenyl)-2(*Z*), 4(*Z*)-heptadienoyl] pyrrolidine) have been tested against promastigotes of *Leishmania amazonensis*, showing promising results [27]. Additionally, a derivative compound was tried against epimastigote and amastigote forms of *T. cruzi* [31]. On the other hand, *P. martensianum* does not have reports of its phytochemical composition nor biological activities, thus, this is the first report of biological activity attributed to this specie. Its promising activity could be related to benzoic acid derivatives and flavonoids as has been demonstrated in other studies of *Piper* species [28, 26, 5].

Casearia genus belongs to Salicaceae family and is traditionally used for gastric disorders, wound healing, and as a topical anesthetic, anti-inflammatory, antiophidic, antipyretic, and antiseptic [33]. Among its species, *C. corymbosa* is the second most active specie against epimastigotes of *T. cruzi*, suppressing the 98.88% of viability at 100 µg/mL, **Table 2**. From its leaves and bark have been isolated some diterpenoids such as clerodane, labdane, kaurene, kolovanetypes, γ-sitosterol, and phenolic acids [34, 35, 36]. Even with its interesting chemical composition, the antitrypanosomal activity of *C. corymbose*-like *Piparea dentata* (previously known as *Casearia commersoniana*)- has not yet been thoroughly investigated. However, another species from the same genus, *Casearia sylvestris,* has been reported to have antitrypanosomal activity against *T. cruzi* [37], supporting its applicability in this type of studies.

The aerial parts extract of *P. dentata* was the most active in this study, suppressing the 98.94% viability of epimastigotes of *T. cruzi* at 100 µg/mL, **Table 2**. This is the first report of biological activity; nevertheless, some reports of goat toxicity of its fruits have been reported [38]. Therefore, it is advisable to carry out toxicity studies on this species before delving into its phytochemical and biological studies. *Solanum nudum* (Solanaceae) fruits (viability suppression at 100 µg/mL:91.64±4.80%, **Table 2**) are used in traditional medicine to treat fevers [39], and several steroids, and sapogenins have been isolated [40, 39, 41, 42]. Some of them presented antiplasmodial or antimalarial activity, and it was determined that none were mutagenic, clastogenic,or cytotoxic [39, 41, 42]. Toxicity studies are recommended, as the unripe fruits of many *Solanum* species exhibit toxicity to humans [43].

CONCLUSION: From 114 tested species, 34 were active against *T. cruzi* epimastigotes, and it is interesting to highlight the prominent activity of 9 species (viability suppression > 90%). Not with standing, *Casearia corymbosa, Peperomia pseudoalpina,* and *Piper martensianum* are the most promising species according to their chemical composition, biological activities, and toxicity. Therefore, these species could be promising sources for isolating new compounds with therapeutic potential against Chagas disease. This confirms that the Salvadoran flora is a rising source of antitrypanosomal species and antikinetoplastid agents.

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