Research Article

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Future Medicinal Chemistry

In vitro antiprotozoal activity of some medicinal plants against sleeping sickness, Chagas disease and leishmaniasis

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Aim: Antiprotozoal activity of 36 medicinal plants was evaluated. **Materials & methods:** *In vitro* potency against *Trypanosoma brucei brucei*, *T. b. rhodesiense*, *T. cruzi* and *Leishmania infantum* beside cytotoxicity on MRC-5 fibroblasts were determined. **Results & conclusion:** *Maytenus parviflora* showed the highest activity against *T. b. brucei* (IC_{50} of 0.6 μg/ml) and *T. b. rhodesiense* (IC_{50} of 0.5 μg/ml) with low cytotoxicity (CC_{50} of 30 μg/ml). *Saussurea costus* and *Commiphora wightii*, showed pronounced potency against *T. cruzi* with an IC_{50} of 3.6 and 2.5 μg/ml, respectively. *Jatropha pelargonifolia* and *Solanum villosum* exhibited pronounced activity toward *L. infantum* with an IC_{50} of 3.2 and 2.0 μg/ml, respectively. *M. parviflora*, *S. costus*, *C. wightii*, *J. pelargonifolia* and *S. villosum* showed relevant selectivity.

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Trypanosoma and *Leishmania* infections are a serious health problem worldwide. Mortality and morbidity due to these protozoa in Asia, Africa and Latin-America are worthy of attention. African trypanosomiasis is caused by *Trypanosoma brucei rhodesiense* and *T. b. gambiense*. *T. b. rhodesiense* is responsible for causing the disease in east and southern Africa [1,2]. Sleeping sickness is a major cause of mortality and morbidity in sub-Saharan Africa and causes serious economic and health problems accounting for 1.5 million disability-adjusted life years, a scale representing the loss of healthy and productive life per year due to disease [3,4]. 60million people are at risk of infection, which is fatal when left without treatment [5,6]. This disease was classified as a category 1 by the Special Program for Research and Training in Tropical Diseases (TDR) of the WHO due to its high rate of re-emergence [4]. WHO report of 16 February 2018, that continuous control efforts have reduced the new cases numbers. In 2009, the reported number dropped below 10,000 for the first time in the last 50 years. In 2012, WHO set a global elimination target to achieve 'fewer than 2000 reported cases per year' by 2020. In 2015, there were 2804 cases recorded. Nowadays there are only four drugs (pentamidine, suramin, eflornithine and melarsoprol) beside nifurtimox–eflornithine combination therapy for the second stage disease approved for treatment [7,8] which requires long parenteral administration and causes frequent and sometimes severe side effects. It is also unaffordable for poor people in third world and developing countries. Additionally, eflornithine and pentamidine are not active against *T. b. rhodesiense*. Several patients develop drug resistance and fail to respond to melarsoprol [9].

More than 28 million humans are at risk of infection by Chagas disease (American trypanosomiasis) caused by *T. cruzi* and 15 million are infected [10]. The disease is endemic in Latin American countries and transmitted to man via contact with Triatome bugs [11]. People infected with chagas disease manage their condition either with nifurtimox or benznidazole, however, not without severe side effects [10].

Leishmaniasis is caused by several *Leishmania* species. Clinical symptoms of leishmaniasis are varying from ulcers in cutaneous leishmaniasis [12] to progressive nasopharyngal involvement in mucocutaneous leishmaniasis or disseminating visceral leishmaniasis (VL) [13,14]. *Leishmania donovani* is particularly prevalent in India and Nepal while *L. infantum* cause infantile VL in the Mediterranean region and Latin America [14]. WHO reported that

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about 12 million people are infected all over the world [15,16]. Antimonials, miltefosine and amphotericin B are drugs of choice. In several undeveloped countries in Africa, Asia and Latin America, treatment may still depend on traditional herbal products, of which the efficiency and safety are uncertain.

There is a need for re-examining several medicinal plants for the antiparasitic activity potential since natural products either from microorganisms or plants represent an unlimited source for novel drugs [13,17]. In this study, we explored the *in vitro* antitrypanosomal and antileishmanial potency of 31 plant species in an attempt to identify possible new antitrypanosomal and antileishmanial 'hits'. The plants were randomly selected for *in vitro* evaluation adopting standard screening procedures [13,18]. The random selection approach was based on our previous experience [19–21].

Materials & methods

Chemicals

Benznidazole, suramin, miltefosine and tamoxifen were used as a reference drug control for *T. cruzi*, *T. b. brucei* and *rhodesiense*, *L. infantum* and MRC-5, respectively. The reference drugs were obtained from WHO-TDR and Sigma-Aldrich (Bornem, Belgium).

Plant material

Totally, 31 plants belonging to 23 families were collected from Arabian Peninsula (Saudi Arabia and Jordan) and Africa (Egypt, Libya and Sudan) between January and February 2014. Random collection of the plants was followed by *in vitro* antiparasitic screening following standard approaches [13]. Voucher samples are kept at the Pharmacognosy Department Herbarium, Faculty of Pharmacy, Cairo University. The collected plants were air-dried, then grounded.

Preparation of plant extracts

Dried powdered plant materials (40 g each) were separately extracted with methanol (4 × 100 ml) using homogenizer. The solvent free methanol extract (ME) was kept for further *in vitro* screening tests at 4°C.

Fractionation of some selected crude extracts

Six crude extracts were selected for further fractionation to verify the effect on the activity. Three active extracts (*Pistacia atlantica*, *Saussurea costus* and *Solanum villosum*) and three inactive ones (*Vicia ervilia*, *Marrubium vulgare* and *Verbascum longibracteatum*) were randomly selected. The ME of the selected plants were suspended in water and then fractionated by consecutive partitioning against methylene chloride (fr-A), and *n*-butanol (fr-B), in addition to fr-C representing the remaining aqueous [22]. All the prepared fractions were re-screened for their *in vitro* antiprotozoal activity.

In vitro biological assay

Standard protocols used by the Lab of Microbiology, Parasitology and Hygiene, Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium were applied [13].

Test plate production

The tests were done in 96-well plates (Greiner, Bio-One, Wemmel, Belgium) [19]. Dilutions were done by a robotic station (BIOMEK 2000, Beckman, CA, USA). All experiments were carried out in duplicate in an independent replicate experiment.

Antitrypanosomal potency

Trypomastigotes of *T. b. brucei* (suramin-sensitive) Squib-427 strain were cultured in Hirumi-9 medium (at 37°C and 5% CO_2) supplemented with 10% fetal calf serum following the procedures described in [23–25]. The *T. b. rhodesiense* assay was performed as described in [26]. Stock solutions of the extracts were prepared according to [27]. Upon the addition of trypomastigotes from an axenic culture to the microtiter plate containing the extract dilutions, it was incubated for 72 h in 5% CO_2 at 37°C. Resazurin (12.5 mg resazurin in 100 ml distilled water) was then added to each well and further incubated for 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorimeter (Molecular Devices Cooperation, CA, USA) using an emission λ 588 nm and excitation λ 536 nm [24].

Trypanosoma cruzi Tulahuen CL2 (nifurtimox-sensitive) strain was maintained on MRC-5 cells and the antitrypanosomal potency on intracellular amastigotes was as described in [12,25] procedures.

Antileishmanial activity

Mouse macrophages were stimulated by intraperitoneal injection of starch. Two days later, the macrophages were collected and seeded (3×10^4) in 96-well plates incubated in 5% CO_2 at 37°C. After 2 days, *ex vivo* (spleen-derived) amastigotes of *L. infantum* (MHOM/FR/96/LEM3323) were used to infect the primary peritoneal murine macrophages (PMM) at a 10:1 infection ratio. The plates were further incubated for 2 h before addition of the sample dilutions. Cells were dried after 5 days of incubation, fixed with methanol and stained with 20% Giemsa for microscopic reading. Results were expressed as percentage reduction of amastigote burden compared with the control untreated cultures.

The potency of the tested samples was assessed according to the mentioned SC. Score 1: inactive; 2: mild; 3: moderate; 4: pronounced; 5: strong; 6: very strong activity.

Cytotoxicity assay

MRC-5 cells (human embryonic lung fibroblasts) or PMM were cultivated in MEM medium or RPMI 1640 (Roswell Park Memorial Institute) medium, respectively, supplemented with L-glutamine (20 mM), 5% FCS and 16.5 mM $NaHCO_3$ (37°C and 5% CO_2). Then, 10^4cells/well were seeded onto the prediluted sample test plates, and then incubated at 5% CO_2 and 37°C for 72 h. Cells viability was determined fluorimetrically after addition of resazurin.

Results

In a continuation of our interest in exploring plants with antiprotozoal potential [19–21,28–30], 31 plants were collected from Arabian Peninsula (Saudi Arabia and Jordan) and Africa (Egypt, Libya and Sudan). The *in vitro* antiprotozoal potency was assessed against *T. cruzi*, *T. b. brucei*, *T. b. rhodesiense* and *L. infantum*, as well as against MRC-5 and PMM cell lines for cytotoxicity and evaluation of selectivity.

Analysis of the results is based on IC_{50}-values (μg/ml), a scoring system (SC) as adopted by Lab of Microbiology, Parasitology and Hygiene was used (Table 1).

Only the extract of *M. parviflora* (aerial parts) showed SC 5 with an IC_{50} of 0.5 μg/ml (SI 56.3) and 0.5 μg/ml (SI 64) against *T. b. brucei* and *T. b. rhodesiense*, respectively, which could represent a promising candidate. Most of the plant extracts had low cytotoxicity (SC 1 or 2), except four extracts showing moderate cytotoxicity (SC 3). The most sensitive protozoa was *T. b. rhodesiense* with eight extracts of SC 3, three with SC 4 and one with SC 5, whereas the most resistant one was *L. infantum*, with only four extracts with SC 3 and two with SC 4.

Cytotoxicity & selectivity

Twenty-four samples were noncytotoxic against MCR5 cells (score 1, $CC_{50}>37$) and eight samples had low cytotoxicity (SC 2, $CC_{50}>13$ μg/ml) (Table 1). Only four extracts showed moderate cytotoxicity (SC 3, $CC_{50}>5$ μg/ml), namely *S. costus* root, *Combretum hartmannianum* stem and bark, and aerial parts of *Jatropha pelargonifolia* with CC_{50} of 6.9, 10.4, 10.5 and 12.9 μg/ml respectively. No further screening of these samples was undertaken with the exception of *S. costus* extract that was further fractionated since Julianti *et al.* [31] reported antitrypanosomal activity of sesquiterpene lactones of *S. costus*. The ME of *M. parviflora* aerial parts (SI 56.3), *Schinus molle* oil resin (SI 31.7) and *Pistacia lentiscus* root oil (SI 30.3) showed high selectivity toward *T. b. brucei* based on their cytotoxicity and IC_{50}. The most selective extracts toward *T. b. rhodesiense* were *M. parviflora* aerial parts (SI 64.0) followed by *P. atlantica* leaves (SI 32.0), *Commiphora myrrha* gum resin (SI 28.0), and *S. molle* oleoresin (SI 22.7). In addition, the ME of *C. wightii* gum resin (SI 9.3), *Solanum diphyllum* root (SI 8.9) and *P. atlantica* leaves (SI 7.4) showed the highest selectivity against *T. cruzi*. *S. villosum* (SI 31.5) followed by *P. atlantica* (SI 7.4) were the most selective against *L. infantum*.

Antitrypanosomal activity

Among all tested plants extracts, the extract of *M. parviflora* aerial parts (family Celesteraceae) showed the most potent activity against *T. b. rhodesiense* (score 5) with an IC_{50} of 0.5 μg/ml, SI 64 and low cytotoxicity score 2. It also exhibited strong activity (score 5) against *T. b. brucei* (IC_{50} 0.5 μg/ml) and moderate activity (score 3) against *L. infantum* ($IC_{50}>10.77$ μg/ml) (Table 1). On *T. b. brucei*, *S. molle* oleoresin, *P. lentiscus* root oil and *Boswellia carterii* gum resin exhibited pronounced activity (score 4) with an IC_{50} of 2.0, 2.1, 2.8 μg/ml and SI 31.7, 30.3, 9.8, respectively. Both *S. molle* and *P. lentiscus* exhibited no cytotoxicity (score 1), whereas *B. carterii* showed low

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Table 1. Antiprotozoal activity of medicinal plants crude extracts and their cytotoxicity against MRC-5 cells.

Plant name	Family	Sample	PC	MRC-5		*Trypanosoma brucei brucei*			*T. b. rhodesiense*			*T. cruzi*			*Leishmania infantum*		
				CC_{50}	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†
Schinus molle L.	Anacardiaceae	Rs.	SWS	22.9	2	8.1	2.8	3	8.1	2.8	3	9.7	2.3	3	32.4	0.7	1
		O.RS.	SWS	>64.0	1	2.0	31.7	4	2.8	22.7	4	30.4	2.1	2	32.4	1.9	1
Pistacia atlantica (Desf.)		L.	Li	>64.0	1	5.2	12.1	3	2.0	32.0	4	8.6	7.4	3	8.6	7.4	3
Pistacia lentiscus L.		R. Oil	HDS	>64.0	1	2.1	30.3	4	6.3	10.1	3	34.8	1.8	1	43.1	1.4	1
Dorema ammoniacum (D. Don)	Apiaceae	G.R.	HDS	24.2	2	9.4	2.5	2	7.9	3.1	3	6.7	3.6	3	12.7	1.9	2
Asparagus officinalis L.	Asparagaceae	A.P.	HDS	>64.0	1	40.3	1.5	1	31.2	2.1	1	29.6	2.1	2	32.4	1.9	1
Cnicus benedictus L.	Asteraceae	A.P.	SWS	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1
Saussurea costus (Falc.)		R.	HDS	6.9	3	25.8	0.2	1	12.2	0.5	2	3.5	1.9	4	7.5	0.9	3
Carduncellus eriocephalus (Boiss.)		A.P.	NCE	>64.0	1	32.6	1.9	1	8.3	7.6	3	>64.0	1.0	1	50.8	1.2	1
Cordia myxa L.	Boraginaceae	F.	SWS	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1
Commiphora wightii (Arn. Bhandari)	Burseraceae	G.R.	HDS	23.2	2	8.1	2.8	3	7.6	3.0	3	2.4	9.3	4	9.5	2.4	3
Commiphora myrrha (Nees Engl.)		G.R.	HDS	>64.0	1	8.1	>7.8	3	2.2	28.0	4	33.2	1.9	1	20.3	3.1	2
Boswellia carterii (Birdw.)		G.R.	HDS	27.6	2	2.8	9.8	4	7.5	3.6	3	7.1	3.8	3	32.4	0.8	1
Maytenus parviflora (Vahl)	Celasteraceae	A.P.	SWS	32.0	2	0.5	56.3	5	0.5	64.0	5	18.6	1.7	2	10.7	2.9	3
Chenopodium schraderianum (Roemer and Schultes)	Chenopodiaceae	A.P. Oil	SWS	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1
Combretum hartmannianum (Schweinf.)	Combretaceae	L.	MES	46.3	1	35.1	1.3	1	9.9	4.6	2	35.4	1.3	1	38.0	1.2	1
		St.	MES	10.4	3	35.8	0.2	1	19.9	0.5	2	22.9	0.4	2	32.4	0.3	1
		B.	MES	10.5	3	35.4	0.3	1	32.5	0.3	1	29.3	0.3	2	32.4	0.3	1
Jatropha pelargonifolia (Courb.)	Euphorbiaceae	A.P.	SWS	12.9	3	28.5	0.4	1	6.9	1.8	3	7.2	1.7	3	3.1	4.0	4
Sophora japonica L.	Fabaceae	F.	ESE	>64.0	1	23.1	2.7	2	7.7	8.2	3	>64.0	1.0	1	>64.0	1.0	1
		St. B.	ESE	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	37.6	1.7	1	>64.0	1.0	1

†**Scores adopted by LMPH (μg/ml) for assessment of antiprotozoal and cytotoxic activities of the crude extracts.** *T. cruzi*, score 1: >33, 2: >1, 3: >4, 4: >1.51, 5: >0.55; *T. brucei brucei*, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; *T. brucei rhod*, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; *L. infantum*, score: >30, 2: >11, 3: >4, 4: >1.51, 5: >0.55, 6>0.2; cytotoxicity, scores: low cytotoxicity: score 1: >37, 2: >13; moderate cytotoxicity: score 3: >5; high cytotoxicity: score 4: >1.8, 5: >0.6; Activity score 1: inactive, 2: mild, 3: moderate, 4: pronounced, 5: strong, 6: very strong.

AG: Aswan governorate; A.P.: Aerial part; CC_{50}: Concentration causing 50% cytotoxicity; EG: Egypt; ESE: Experimental station of Faculty of Pharmacy, Cairo, Egypt; F: Fruit; F.P.: Fruit peel; G.R.: Gum resin; HDS: Herbal drug store, Cairo, Egypt; IC_{50}: Concentration causing 50% inhibition; JO: Jordan; L: Leave; Li: Libya; LMPH: Lab of Microbiology, Parasitology and Hygiene; MES: Mid-East of Sudan; MRC-5: Diploid human embryonic lung fibroblast; Nc: Not completed; NCE: North coast, Egypt; O. Rs.: Oil resin; PC: Place of collection; R: Root; R.B.: Root bark; Rs.: Resin; SC: Score; Sc.: Scale; Se.: Seed; SI: Selectivity index; St.: Stem; St. B.: Stem bark; SWS: Southern and western areas of Saudi Arabia; VM: Vegetable market; ZG: Zoo garden.

Table 1. Antiprotozoal activity of medicinal plants crude extracts and their cytotoxicity against MRC-5 cells (cont.).

Plant name	Family	Sample	PC	MRC-5		*Trypanosoma brucei brucei*			*T. b. rhodesiense*			*T. cruzi*			*Leishmania infantum*		
				CC_{50}	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†
Vicia ervilia Willd.		A.P.	JO	>64.0	1	>64.0	1.0	1	32.5	1.9	1	>64.0	1.0	1	>64.0	1.0	1
Marrubium vulgare L.	Labiatae	A.P.	SWS	59.1	1	33.1	1.7	1	9.9	5.9	2	36.5	1.6	1	32.4	1.8	1
Allium cepa (red cultivar) L.	Liliaceae	Sc.	VM	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1
Phragmanthera austroarabia A.G. (Mill.)	Loranthaceae	A.P.	SWS	20.8	2	33.1	0.6	1	37.6	0.5	1	22.3	0.9	2	32.4	0.6	1
Eucalyptus maculate Hook.	Myrtaceae	St. B.	ZG	>64.0	1	37.2	1.7	1	34.4	1.8	1	33.8	1.8	1	>64.0	1.0	1
Jasminum grandiflorum L.	Oleaceae	A.P.	SWS	27.8	2	31.2	0.8	1	28.3	0.9	1	29.4	0.9	2	24.0	1.1	2
Cistanche phelipaea L. (P. Cout.)	Orobancheae	F.	SWS	>64.0	1	32.5	1.9	1	32.9	1.9	1	34.1	1.8	1	>64.0	1.0	1
Oryza sativa L. (Bran)	Poaceae	R.B.	EG	>64.0	1	33.1	1.9	1	27.5	2.3	1	35.8	1.7	1	>64.0	1.0	1
Prunus korshinskyi (Hand. Mazz.)	Rosaceae	L.	SWS	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	32.0	2.0	1	32.4	1.9	1
Citrus aurantium L.	Rutaceae	F.P.	EG	>64.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1	>64.0	1.0	1
Verbascum longibracteatum (Deflers.)	Scrophulariaceae	A.P.	SWS	29.0	2	>64.0	0.4	1	43.0	0.6	1	28.1	1.0	2	32.4	0.9	1
Solanum diphyllum L.	Solanaceae	L.	AG	>64.0	1	33.1	1.9	1	28.7	2.2	1	40.3	1.5	1	>64.0	1.0	1
		R.	AG	>64.0	1	8.3	7.6	3	12.2	5.2	2	7.1	8.9	3	>64.0	1.0	1
Solanum villosum (Miller)		A.P.	ESE	>64.0	1	>64.0	1.0	1	31.5	2.0	1	32.0	2.0	1	2.0	31.5	4
Tamarix aphylla L.	Tamaricaceae	A.P.	SWS	>64.0	1	>64.0	1.0	1	52.3	1.2	1	>64.0	1.0	1	>64.0	1.0	1
Standards:																	
– Tamoxifen				9.3		Nc			Nc			Nc			Nc		
– Benznidazole				Nc		Nc			Nc			2.6			Nc		
– Suramin				Nc		0.04			0.04			Nc			Nc		
– Miltefosine				Nc		Nc			Nc			Nc			10.7		

†**Scores adopted by LMPH (μg/ml) for assessment of antiprotozoal and cytotoxic activities of the crude extracts.** *T. cruzi*, score 1: >33, 2: >1, 3: >4, 4: >1.51, 5: >0.55; *T. brucei brucei*, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; *T. brucei rhod*, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; *L. infantum*, score: >30, 2: >11, 3: >4, 4: >1.51, 5: >0.55, 6>0.2; cytotoxicity, scores: low cytotoxicity: score 1: >37, 2: >13; moderate cytotoxicity: score 3: >5; high cytotoxicity: score 4: >1.8, 5: >0.6; Activity score 1: inactive, 2: mild, 3: moderate, 4: pronounced, 5: strong, 6: very strong.

AG: Aswan governorate; A.P.: Aerial part; CC_{50}: Concentration causing 50% cytotoxicity; EG: Egypt; ESE: Experimental station of Faculty of Pharmacy, Cairo, Egypt; F: Fruit; F.P.: Fruit peel; G.R.: Gum resin; HDS: Herbal drug store, Cairo, Egypt; IC_{50}: Concentration causing 50% inhibition; JO: Jordan; L: Leave; Li: Libya; LMPH: Lab of Microbiology, Parasitology and Hygiene; MES: Mid-East of Sudan; MRC-5: Diploid human embryonic lung fibroblast; Nc: Not completed; NCE: North coast, Egypt; O. Rs.: Oil resin; PC: Place of collection; R: Root; R.B.: Root bark; Rs.: Resin; SC: Score; Sc.: Scale; Se.: Seed; SI: Selectivity index; St.: Stem; St. B.: Stem bark; SWS: Southern and western areas of Saudi Arabia; VM: Vegetable market; ZG: Zoo garden.

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cytotoxicity (score 2). Five tested crude extracts showed moderate activity (score 3) with an IC_{50}>3 μg/ml, whereas only two tested samples were of mild activity (score 2) with an IC_{50}>9 μg/ml.

Again *M. parviflora* aerial parts ME demonstrated strong activity (score 5) with an IC_{50} of 0.5 μg/ml, SI 64.0 and low cytotoxicity score 2. The *P. atlantica* leaves ME, *Commiphora myrrha* gum resin and *S. molle* oil resin showed pronounced activity (score 4) with an IC_{50} of 2.0, 2.2 and 2.8 μg/ml and SI 32.0, 28.0, 22.7, respectively. Eight crude extracts (22.22%) showed moderate activity (score 3) with an IC_{50}>4 μg/ml, whereas five samples showed mild activity (score 2) with an IC_{50}>11 μg/ml. The *C. wightii* gum resin demonstrated pronounced activity (score 4) with an IC_{50} of 2.4 μg/ml, SI 9.3 with low cytotoxicity score 2 followed by *S. costus* root ME (IC_{50} of 3.5, 2.4 μg/ml and SI 1.96) that exhibited moderate cytotoxicity score 3. Six crude extracts showed moderate activity (score 3) with an IC_{50}>4 μg/ml, whereas eight crude extracts were of mild activity (score 2) with an IC_{50}>11 μg/ml.

Antileishmanial activity

The ME of *S. villosum* and *J. pelargonifolia* aerial parts exhibited pronounced activity (score 4) against *L. infantum* (Table 1), with an IC_{50} of 2.03 and 3.17 μg/ml and SI of 31.55 and 4.08, respectively. *S. villosum* exerted a cytotoxic effect with IC_{50} of 2.0 μg/ml (score 4) while *J. pelargonifolia* exhibited no cytotoxicity against PMM cell line. Four tested crude extracts showed moderate activity (score 3) with an IC_{50} >4 μg/ml, whereas three tested crude extracts were of mild activity (score 2) with an IC_{50} >11 μg/ml.

Effect of crude extracts fractionation

Random selection of three active and three inactive extracts was applied for further fractionation to verify the effect of fractionation on the activity. These plants were *P. atlantica*, *S. costus*, *S. villosum*, *V. ervilia*, *M. vulgare* and *V. longibracteatum*. Five of the selected plants were found to be of no or low cytotoxicity (score 1 or 2) and only *S. costus* was of a moderate cytotoxicity (SC 3).

Fractionation caused significant increase in the antiprotozoal activity of the aforementioned plants except for *P. atlantica* (Table 2). Fr-B of *S. villosam* showed very strong activity (score 6) against *L. infantum* with an IC_{50} of 0.5 μg/ml (however, accompanied by cytotoxicity on PMM with an IC_{50} of 0.5 μg/ml). In addition, a pronounced activity (score 4) was recorded against *T. b. rhodesiense* with an IC_{50} of 2.4 μg/ml and a moderate activity (score 3) against *T. b. brucei* and *T. cruzi* with an IC_{50} of 8.3 and 8.1 μg/ml, respectively. This potent antiprotozoal activity of fr-B is accompanied with high selectivity in case of *L. infantum* (SI 51.68) and moderate selectivity for *T.b. rhodesiense* (SI 10.53). Also, fr-A displayed moderate activity (score 3) against *T. b. rhodesiense* (IC_{50} of 8.0 μg/ml). It is worthy pointing out that *S. villosam* crude extract was completely inactive against all tested *Trypanosoma* species. The activity of *S. costus* (fr-A) against *T. b. brucei*, *T.b. rhodesiense* and *L. infantum* (IC_{50} of 8.1, 7.4 and 2.0 μg/ml, respectively) was much higher than the activity of the crude ME with the same level of cytotoxicity (score 3). *S. costus* (fr-B) revealed high activity against tested protozoa (scores 3–5) higher than that of the total ME yet it exhibited higher cytotoxicity (score 5).

Upon fractionation of the inactive ME of *V. ervilia* seeds, it was noticed that fr-B and fr-A exhibited mild-to-moderate antitrypanosomal activity (scores 2 and 3). In the same vein, fr-B of the inactive *V. longibracteatum* aerial parts ME showed mild-to-moderate activity (scores 2 and 3) against *T. b. rhodesiense* and *L. infantum*. Also, fractionation of the crude ME of *M. vulgare* against *T.b. rhodesiense* lead to increase in the potency from score 2 to score 3 (fr-A). On the other hand, our findings indicated that all *P. atlantica* fractions were inactive (score 1) against all tested protozoa while its total crude ME exhibited moderate (score 3) to pronounced (score 4) activity.

Discussion

The most promising activity was demonstrated by *M. parviflora* against *T. b. brucei* and *T. b. rhodesiense*. Nothing was found in the literature regarding the phytochemical constituents of *M. parviflora*, except the study conducted in the Kingdom of Saudi Arabia that reported the presence of flavonoid, phenol, saponin, alkaloid, triterpenoid and anthraquinone [32]. It was reported that several anthraquinone [33] and alkaloid derivatives [34,35] possess antitrypanosomal and antileishmanial activity. It was found that some alkaloids intercalate DNA target the sensitive kDNA of the trypanosomes [34,35]. The possible presence of anthraquinone and alkaloid content [32] (from results of phytochemical screening,unpublished data) of Maytenus parviflora may explain it spotent activity against T. b. brucei and T. b. rhodesiense.

Table 2. Antiprotozoal activity of subfractions obtained from randomly selected active and inactive extracts.

Plant name	Fraction	MRC-5		*T. b. brucei*			*T. b. rhodesiense*			*T. cruzi*			*Leishmania infantum*		
		CC_{50}	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†	IC_{50}	SI	SC†
Active plant fractions															
Pistacia atlantica (Desf.)	Fr-A	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
	Fr-B	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
	Fr-C	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
Saussurea costus (Falc.)	Fr-A	7.1	3	8.1	0.8	3	7.4	0.9	3	2.2	3.2	4	2.0	3.5	4
	Fr-B	1.6	5	8.0	0.2	3	7.9	0.2	3	0.5	2.8	5	2.0	0.8	4
	Fr-C	26.3	2	>64.0	0.4	1	>64.0	0.4	1	25.4	1.0	2	32.4	0.8	1
Solanum villosum (Miller)	Fr-A	13.8	2	30.8	0.4	1	8.0	1.7	3	30.1	0.4	2	32.4	0.4	1
	Fr-B	26.2	2	8.3	3.1	3	2.4	10.5	4	8.1	3.2	3	0.5	51.6	6
	Fr-C	>64.0	1	>64.0	>1.0	1	32.4	>1.9	1	>64.0	1.0	1	32.4	>1.9	1
Inactive plant fractions															
Vicia ervilia (Willd.)	Fr-A	>64.0	1	24.4	>2.6	2	16.9	>3.7	2	61.5	1.0	1	50.8	>1.2	1
	Fr-B	>64.0	1	26.4	>2.4	1	7.3	>8.6	3	29.8	2.1	2	32.4	>1.9	1
	Fr-C	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
Marrubium vulgare L.	Fr-A	30.2	2	32.9	0.9	1	7.6	3.9	3	36.1	0.8	1	32.4	0.9	1
	Fr-B	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
	Fr-C	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	>64.0	1.0	1	>64.0	>1.0	1
Verbascum longibracteatum (Deflers.)	Fr-A	>64.0	1	>64.0	>1.0	1	>64.0	>1.0	1	33.4	1.9	1	>64.0	>1.0	1
	Fr-B	>64.0	1	>64.0	>1.0	<1	17.4	>3.6	= 2	39.5	1.6	1	9.5	>6.7	3
	Fr-C	>64.0	1	>64.0	>1.0	<1	>64.0	>1.0	<1	>64.0	1.0	1	>64.0	>1.0	1
Standards															
Tamoxifen		9.3		Nc			Nc			Nc			Nc		
Benznidazole		Nc		Nc			Nc			2.6			Nc		
Suramin		Nc		0.04			0.04			Nc			Nc		
Miltefosine		Nc		Nc			Nc			Nc			10.7		

†Scores adopted by LMPH (μg/ml) for assessment of antiprotozoal and cytotoxic activities of the crude extracts. T. cruzi, score 1: >33, 2: >1, 3: >4, 4: >1.51, 5: >0.55; T. brucei brucei, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; T. brucei rhod, score 1: >24, 2: >9, 3: >3, 4: >1.2, 5: >0.4; L. infantum, score: >30, 2: >11, 3: >4, 4: >1.51, 5: >0.55, 6>0.2; cytotoxicity, scores: low cytotoxicity: score 1: >37, 2: >13; moderate cytotoxicity: score 3: >5; high cytotoxicity: score 4: >1.8, 5: >0.6; Activity score 1: inactive, 2: mild, 3: moderate, 4: pronounced, 5: strong, 6: very strong.

CC_{50}: Concentration causing 50% cytotoxicity; Fr-A: Methylene chloride; Fr-B: *N*-butanol; Fr-C: Aqueous remaining fraction; IC_{50}: Concentration causing 50% inhibition; MRC-5: Normal human fetal lung fibroblast cell line; Nc: Not completed; SC: Score; SI: Selectivity index.

In another study, *M. senegalensis* showed strong activity against *Plasmodium falciparum* [36] that encourage further study of the antiparasitic activity of this plant.

It was noted that extracts of several plants under investigation, namely, *S. molle*, *P. atlantica*, *C. wightii*, *B. carterii* and *Solanum diphyllum* exhibited moderate to pronounced potency against both *T. brucei* and *T. cruzi*, which is in agreement with findings of [10] who reported that many natural agents which have been examined against *T. brucei* also affected *T. cruzi*. The results revealed that the most active plant against *T. cruzi* was *C. wightii* followed by *S. costus*. The most possible cause of their pronounced activity against *T. cruzi* is their content of quinonoid components [37]. It has been reported that quinonoids [38,39] and sesquiterpene lactones [31] in both plants exhibit strong activity against *T. cruzi*.

The ME of *J. pelargonifolia* and *S. villosum* exhibited pronounced activity against *L. infantum*. Nothing was found in the literature regarding the chemical or biological investigation of *J. pelargonifolia.* In a review on the active constituents isolated from certain plants used traditionally for leishmaniasis treatment, two terpenes: jatrogrossidione isolated from *J. grossidentata* and jatrophone from *J. isabellii*, are among the potent molecules described [40,41]. In another study, the *in vivo*, antileishmanial activity of jatrophone from *J. isabelli* was reported. This compound is active against the virulent strains of *Leishmania*, but reported to be toxic for clinical use [42]. Several diterpenes were isolated from the genus *Jatropha* showing antiplasmodial activity [43–45]. Further studies on *J. pelargonifolia* for the isolation of the active constituents and evaluation of *in vivo* efficacy and safety are highly recommended. The herein reported pronounced antileishmanial activity of *S. villosum* cultivated in Egypt is in accordance with findings of [19] who studied the same plant species collected from Saudi Arabia. Among *Solanum* species, *S. arboreum* Dunal demonstrated leishmanicidal activity [46]. Abdel-Hamid *et al.* [47] initiated a study for the isolation of the phytoconstituents of the *n*-butanol fraction of *S. villosum*, which gave positive test for saponin and alkaloid in preliminary screening. The study resulted in isolation of two furanostanol saponin glycosides, a known glycoside borivilianoside D, and a new glycoside (3β, 5α, 25R)-26-(*β*-D-glucopyranosyloxy) furost-20 (22)-en-3-yl-O-*β*-D-glucopyranosyl-(1→2)]-O-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside, namely villososide [47]. Thus, the potency of n-butanol fraction of *S. villosum* could be attributed to their content of steroidal glycosides [48] or steroidal alklaoids [49,50].

Our study revealed an additional four potent antileishmanial medicinal plants to be added to the traditionally used medicinal plants for *Leishmania* treatment worldwide. Most of the tested plant extracts were less active than the drugs used against *L. infantum* except for *P. atlantica*, *C. wightii*, *M. parviflora* and *S. villosum*. Their extracts showed antileishmanial activities with IC_{50} of 8.6, 9.5, 10.7 and 2.0 that roughly compares to the standard drug miltefosine IC_{50} (10.7 μg/ml). It is noteworthy to mention that the aforementioned plants are of no or low cytotoxicity. The crude extracts are composed of several different constituents, and the potent compounds might show higher potency in their less complex form. For example, the *V. longibracteatum* and *S. villosum* ME showed an antileishmanial activity with IC_{50} of 32.46 and 2.03 μg/ml, respectively (Table 1). On further fractionation, their *n*-butanol fractions showed activity against *L. infantum* with an IC_{50} of 9.5 and 0.5 μg/ml (Table 2), respectively, less than that of the reference drug miltefosine (IC_{50} of 10.7 μg/ml), which may indicate a chance for isolation of more active constituents.

Saussurea costus (fr-B) showed strong activity against tested protozoa (SC 3–5), which is accompanied by high cytotoxicity (SC 5). Thus, fr-B of *S. costus* exhibits potent antiprotozoal activity but is strongly related to its high cytotoxicity.

The biologically active constituents of *V. ervilia, M. vulgare* and *V. longibracteatum* acting in their less complex fractionated form rather than acting collectively in the total crude ME. Further detailed study on the aforementioned plant species for isolation of their bioactive compounds is on track. The absence of activity of *P. atlantica* different tested fractions might be due to the synergestic effect of *P. atlantica* constituent in the total ME rather than acting fractionated.

Conclusion

In the present study, *in vitro* activities of several plant extracts against protozoan parasites are reported. The most active extracts were from *M. parviflora*, *S. costus*, *C. wightii*, *J. pelargonifolia* and *S. villosum*. Although *P. atlantica* is not the most active plant, it showed high selectivity toward all tested protozoa and no cytotoxicity. Yet all the active samples demonstrated nonspecific activity. Translation of some *in vitro* results into *in vivo* follow-up studies is recommended in the future.

Future perspective

Natural antiprotozoal agents continue to be an important approach in exploring new treatments and result in controlling sleeping sickness, Chagas disease and leishmaniasis due to the rapid development of protozoan resistance to the synthetic drugs. The expansion of screening of medicinal plants for their antiprotozoal activity will greatly help in finding new agents with higher efficacy and better safety profile. These newly discovered potent antiprotozoal agents from medicinal plants in this study and other future studies could impact human medicine positively in the future as they demonstrated very promising activity and acceptable safety profile that may permit future progression to further trials for their evaluation in animals and human trials.

Summary points

- Trypanosomiasis and leishmaniasis are a major health problem worldwide.
- *In vitro* screening of the antiprotozoal activity of 31 medicinal plants was carried out.
- The plant extracts were tested against three *Trypanosoma* and one *Leishmania* species.
- The plant extracts were tested for their cytotoxicity against MRC-5.
- *Maytenus parviflora* showed the highest activity against *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*.
- *Saussurea costus* and *Commiphora wightii*, showed strong activity against *Trypanosoma cruzi*.
- *Jatropha pelargonifolia* and *Solanum villosum* exhibited strong activity toward *Leishmania infantum*.
- *Solanum villosum* showed the best selectivity index toward *L. infantum*.

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Ethical conduct of research

The use of laboratory rodents was carried out in strict accordance to all mandatory guidelines (European Union directive 2010/63/EU on the protection of animals used for scientific purposes and the Declaration of Helsinki) and was approved by the ethical committee of the University of Antwerp (UA-ECD 2015-90).

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