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Harnessing nature: a systematic exploration of *in vitro* antileishmanial and antihuman African trypanosomal properties in traditional medicinal plants and their active principles

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ABSTRACT

Context: Current treatments for leishmaniasis and Human African Trypanosomiasis (HAT) are constrained by toxicity, high costs, and the growing threat of parasite resistance. In this context, natural products have emerged as promising alternatives, offering the potential for safer and more cost-effective therapeutic interventions.

Objective: This review assesses the antileishmanial and antitrypanosomal activities of medicinal plants, underscoring their significance in addressing parasitic diseases and identifying promising candidates for future drug discovery and development.

Methods: Following the PRISMA 2020 guidelines, a systematic search was conducted for literature published between 2003 and 2023. Studies were considered eligible if they reported *in vitro* activities of extracts or compounds against *Leishmania* spp. or *Trypanosoma brucei* with IC_{50} < 10 µg/mL. Data included plant species, extraction methods, parasite strains, active compound(s), cytotoxicity profiles, and traditional uses. The quality of each study was evaluated based on the reproducibility of experimental procedures.

Results and conclusions: A total of fifty eligible articles were included. 217 plants demonstrated potent activity (IC_{50} < 10 µg/mL) against *Leishmania* spp. and/or *T. brucei*. Among these, 41 species had documented traditional use in the treatment of leishmaniasis or HAT, whereas 76 were traditionally employed for unrelated ailments yet exhibited scientific evidence of antiparasitic efficacy. Notably, 67 species were active against both parasites, 120 displayed selective antitrypanosomal, and 30 showed selective antileishmanial activities. Exemplary candidates include *Achillea ptarmica* L. (pellitorine), *Allium sativum* L. (allicin, ajoene), *Strychnos spinosa* Lam. (triterpenoids), *Tridax procumbens* L. (oxylipin), and *Marrubium incanum* Desr. (salvigenin). More studies should define mechanisms and *in vivo* efficacy.

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Leishmanicidal; trypanocidal; medicinal plants; natural products; antiparasitic compounds

Introduction

Human African Trypanosomiasis (HAT, also known as African sleeping sickness) and leishmaniasis are categorized as Neglected Tropical Diseases (NTDs) by the World Health Organization (WHO) due to the negligence by a large part of the pharmaceutical industry. NTDs primarily affecting poor

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people in tropical and subtropical regions received less attention and were almost ignored by global funding agencies (Engels and Zhou 2020). These diseases are caused by Kinetoplastids organisms, which comprise a category of flagellated protozoans, encompassing organisms such as *Trypanosoma* and *Leishmania* (Stuart et al. 2008).

HAT is fatal if left untreated. The initial stage is characterized by fever, headache, joint pain and pruritus, wit host immune responses actively countering parasite growth (Stuart et al. 2008). These symptoms typically emerge a few weeks after infection. The second stage involves invasion of the central nervous system (CNS), leading to severe neurological manifestations (Stuart et al. 2008). HAT is endemic in sub-Saharan Africa, where tsetse flies transmit the *Trypanosoma brucei* parasite. Two subspecies, *T. brucei gambiense* and *T. brucei rhodesiense,* cause chronic and acute forms of the disease in humans, respectively Frezza et al. 2020). In contrast, *T. b. brucei* vervet monkey models have served as an alternative laboratory system for evaluating HAT, as infected monkeys exhibit clinical, parasitological, and haematological profiles comparable to those infected with *T. b. rhodesiense*. This approach enhances laboratory safety by reducing the risk of accidental infection associated with handling human-infective subspecies (*T. b. gambiense* and *T. b. rhodesiense*) (Waema et al. 2014). Therefore, in the search for therapeutic agents against HAT, both human-infective and non-infective forms of *T. brucei* are relevant and have been considered in this review.

Tsetse flies, the vectors of human African trypanosomiasis (HAT), are distributed across 36 African countries (Frezza et al. 2020). They acquire infection by feeding on the blood of an infected human or animal, after which the parasite undergoes developmental transformations within the fly's alimentary tract and salivary glands before becoming infective. The precise pathogenesis, particularly during the second stage of disease, remains incompletely understood but is thought to involve host immune processes and parasite-derived bioactive molecules (Stuart et al. 2008). Depending on its life cycle stage (Figure 1a), *T. brucei* exhibits several distinct morphological forms: the procyclic form in the tsetse midgut, the epimastigote followed by the metacyclic form in the salivary glands, and the slender from followed by stumpy form in mammalian blood (Matthews 2005).

Leishmaniasis, caused by more than 21 species of *Leishmania*, manifests as a diverse spectrum of illnesses (Stuart et al. 2008). These range from self-healing cutaneous leishmaniasis (CL) to severe and potentially fatal visceral leishmaniasis (VL, or kala-azar). Additional forms include disfiguring mucosal leishmaniasis (ML), diffuse CL characterized by numerous nodular lesions, and post-kala-azar dermal leishmaniasis. VL, primarily attributed to *Leishmania infantum* or *Leishmania donovani*, affects an estimated 200,000-500,000 individuals globally, while CL, caused by various *Leishmania* spp., accounts for more than 5 million cases worldwide (Stuart et al. 2008). The parasite is broadly categorized into Old World and New World leishmaniasis. Old World leishmaniasis is prevalent in Africa, Asia, the Mediterranean, and the Middle East. Some infections remain subclinical and only progress to disease when host immune responses are compromised. Recovery times vary considerably among species. Transmission occurs through the bite of infected female phlebotomine sand flies. Depending on its life cycle stage (Figure 1b), *Leishmania* exhibits two distinct morphological forms: promastigotes in the gut of the sand fly vector and amastigotes within the macrophages of the mammalian host (Hassan et al. 2022).

Hence, in the pursuit of addressing HAT and leishmaniasis, a range of known medications exist for these conditions. However, the available treatments are limited and often come with many side effects. The treatments for HAT include pentamiding, suramin, melarsorol (Venturelli et al. 2022). These medications pose a significant challenge due to their associated adverse effects. For instance, pentamidine may induce hypotension, glucose metabolism abnormalities and renal dysfunction, while suramin is linked to peripheral neuropathy, anaphylactic reactions and bone marrow toxicity (Venturelli et al. 2022). Melarsoprol, in particular, exhibits high toxicity with potentially life-threatening harmful effects such as cardiotoxicity and agranulocytosis (Venturelli et al. 2022). Turning to leishmaniasis, medications like pentavalent antimonials (SbV compounds), amphotericin B, and miltefosine are commonly employed (Hassan et al. 2022). Pentavalent antimonials cause severe side effects such as abdominal pain, erythema and toxicity. Amphotericin B, although effective, is associated with notable side effects, including hypokalemia, anorexia, leukopenia, kidney failure and heart problems (Hassan et al. 2022). On the other hand, miltefosine caused nausea, vomiting, diarrhea and raised creatinine

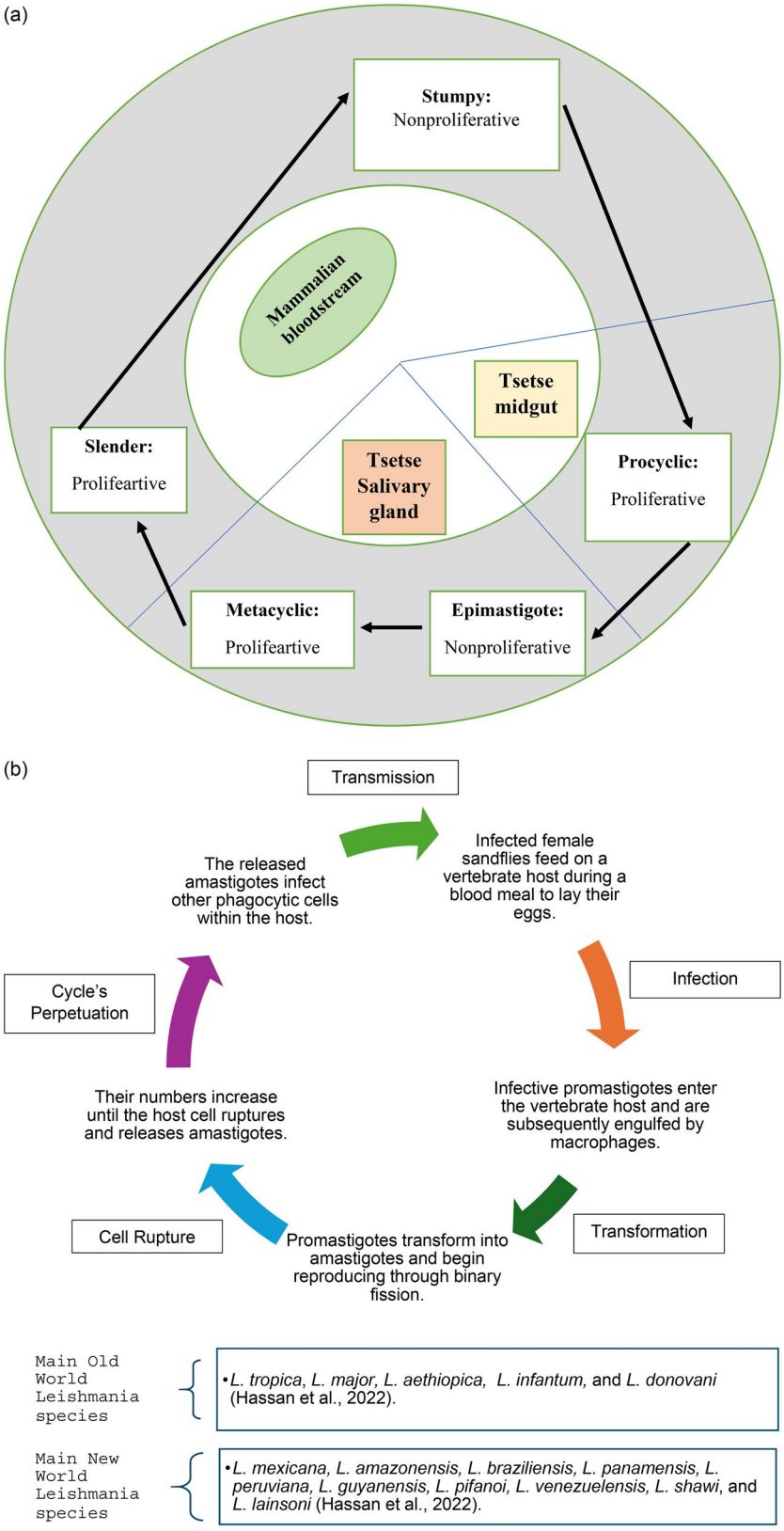

Figure 1. (a) Infection cycle of *Trypanosoma brucei*. Adapted from Matthews (2005). (b) Infection cycle and species of *Leishmania* parasites. Adapted from Hassan et al. (2022).

levels. Most of these medications are typically administered through parenteral or intramuscular routes, except for miltefosine, which is taken orally (Hassan et al. 2022). Thus, the administration of these drugs parenterally or intramuscularly poses challenges for patients due to the inconvenience

and skill required. Moreover, the limited and severe side effects of these medications often lead to noncompliance among patients, contributing to the development of medication-resistant parasites. Consequently, medicinal plants emerge as a viable alternative to conventional treatments for parasitic diseases, given their affordability and perceived safety.

Natural products are treasured sources for discovering and developing safe and valuable medicines against various chronic diseases (Rahmawati et al. 2025). Roughly 70% of the drugs currently in existence find their origins in natural products, predominantly sourced from plants (Mahmood et al. 2014; Jain et al. 2016). The screening of plant extracts containing natural products holds the exciting promise of unveiling new avenues for the discovery of potential drug leads (Yob et al. 2011; Jain et al. 2016). They contain various compounds that can be used for therapeutic purposes to treat many diseases, including leishmaniasis and trypanosomiasis. Natural products have been used for many years to treat these diseases as they are considered safe and affordable compared to the medications on the market. Therefore, this study aims to discover the traditional plants with strong antileishmanial and antitrypanosomal activity for treating leishmaniasis and HAT, respectively.

Materials and methods

Search strategy

Literature about medicinal plants with antitrypanosomal and antileishmanial activity was collected online from published articles using the related keywords from 2003 to 2023. These keywords were entered into the scientific databases, such as PubMed, Scopus and Google Scholar. The articles found and obtained were included based on the reliability of their sources. Some articles were found through cross-referencing as well as by examining the bibliography of other articles. The keywords that were used to obtain required information included 'Natural Product', 'Medicinal Plant', 'Traditional Medicinal Plants', 'Antitrypanosomal', 'Trypanocidal', 'Antileishmanial', 'Leishmanicidal', '*Trypanosoma brucei*', '*Leishmania*', 'Trypanosomiasis', 'Human African Trypanosomiasis', 'Sleeping sickness', 'Leishmaniasis', 'Trypanosomiasis', 'Human African Trypanosomiasis', 'Sleeping sickness', 'Leishmaniasis', 'Antitrypanosomal activity of medicinal plants against *Trypanosoma brucei*', 'Antitrypanosomal activity of medicinal plants against *Leishmania* parasite', 'Antileishmanial activity of medicinal plants against *Trypanosoma* parasite' and 'Antileishmanial activity of medicinal plants against *Leishmania* parasite'.

Inclusion and exclusion criteria

The documents used were chosen based on various criteria: (a) the articles that were published in English, (b) the performed research to screen out antitrypanosomal and antileishmanial activity of medicinal plants against *Trypanosoma brucei* and *Leishmania* parasites, respectively (c) mentioned the half maximal inhibitory concentration (IC_{50}) of the extract(s) or isolated compound(s) (if any) considering the antitrypanosomal and antileishmanial activity, (d) since large quantity of plants exhibited antitrypanosomal and antileishmanial effects, only plants that have IC_{50} value < 10 µg/mL were reported herein. (e) mentioned the cytotoxicity of the extract(s) or isolated compound(s) (if any). Additionally, literature that failed to meet these criteria was specifically excluded when the IC_{50} values were > 10 µg/mL and not included in the Master Journal List. The review articles were also excluded from this study (Figure S1) (Supplementary file).

Data extraction

The information such as the species of the plants, the type of extraction techniques, the active compound(s) if isolated, the strain of *Trypanosoma* and *Leishmania* tested/evaluated, the 50% inhibition concentration (< 10 µg/mL), the cytotoxicity of the extracts and the name of the authors were extracted from relevant literature and presented in the form of a table.

Risk of bias assessment

The Quality Assessment Tool For *in vitro* Studies (QUIN Tool) was considered to evaluate the risk of bias in the included studies (Sheth et al. 2024). This tool was developed due to the lack of a specialized tool to comprehensively evaluate the quality of *in vitro* studies. While various tools exist for assessing the risk of bias in different study designs, none were directly tailored for *in vitro* investigations. Therefore, this tool was developed to guide the quality assessment for *in vitro* studies (Shah 2022). In the evaluation process, reviewers assigned scores to each item in every study, categorizing them as adequately specified (score = 2), inadequately specified (score = 1), not specified (score = 0), or not applicable (excluded from the calculation). The risk of bias was then assessed using twelve criteria and was calculated using this formula: Final Score = (Total score × 100)/(2 × number of criteria applicable). The final scores were used to grade the *in vitro* studies as having a low (>70%), medium (50-70%), or high (<50%) risk of bias. Moreover, the Joanna Briggs Institute (JBI) Checklist for Qualitative Research and Quasi-Experimental Studies was used to measure the risk of bias of the included articles that studied ethnomedicine or studied on plants that had been used traditionally by the folks in affected countries or regions to treat leishmaniasis and HAT (Lockwood et al. 2015; Tufanaru et al. 2020). Reviewers assessed the articles by assigned scores as being 'yes' or 'no' or 'unclear' or 'not applicable'. Any disagreements were resolved through discussion.

Result and discussion

The database search produced a total of 663 articles. After adjustment for duplicates and eligibility criteria, a total of 50 articles were found appropriate for this review. Based on the literature that had been selected, a total of 217 plants were identified for their robust antileishmanial and/or antitrypanosomal activities against two specific parasites namely *Leishmania* and/or *Trypanosoma brucei*. Among them, it was found that 18.9% (41) of the plants were traditionally used by folk and scientifically proven for their antiparasitic effects in treating HAT and leishmaniasis. In contrast, the remaining 81.1% (176) of the plants were traditionally used by folk for various ailments, excluding HAT and leishmaniasis, however they had scientifically been demonstrated to have strong antitrypanosomal and/or antileishmanial effects for the treatment of HAT and leishmaniasis (Figure S2). This noteworthy observation emphasizes that most traditional plants were not initially used to treat HAT and leishmaniasis. However, they were discovered to exhibit substantial potential as alternative treatments that are not only safe but also cost-effective compared to conventional medications, which are associated with high financial burden and side effects. This shows the potential existence of traditional plants with therapeutic properties, not only for other diseases but also for HAT and leishmaniasis. In summary, 67 traditionally used plants demonstrate efficacy against both *Leishmania* and *Trypanosoma* parasites (Figure S3). This illustrates the potential of these traditional medicinal plants to have broad-spectrum activity toward protozoan parasites. Additionally, 120 plants showcase robust antitrypanosomal activity, while 30 plants exhibit potent antileishmanial effects. This highlights that most plants manifest significant effects on *Trypanosoma brucei* parasite compared to *Leishmania* parasite.

Moreover, as illustrated in Figure 2, an interesting finding emerges, revealing that 48.8% (20) out of the 41 plants that were traditionally used to treat HAT and leishmaniasis have been scientifically validated to possess strong antitrypanosomal and antileishmanial activities against *Trypanosoma brucei* and *Leishmania* parasites. Furthermore, a breakdown indicates that 39% (16) of these plants exclusively exhibit potent antitrypanosomal effects, while only 12.2% (5) manifest strong antileishmanial effects. Intriguingly, among the 176 plants that were not traditionally employed for treating leishmaniasis and HAT, a total of 47 (26.7%) have been scientifically proven to demonstrate both strong antileishmanial and antitrypanosomal effects against *Leishmania* and *Trypanosoma brucei* parasites (Figure S4). Conversely, 59.1% (104) of plants have been scientifically verified to exhibit potent antitrypanosomal effects against *Trypanosoma brucei* parasites, while a modest 14.2% (25) were confirmed to possess exclusive antileishmanial activity against *Leishmania* parasite (Figure S4). This highlights a significant trend, indicating that a majority of traditionally used plants that were not initially used

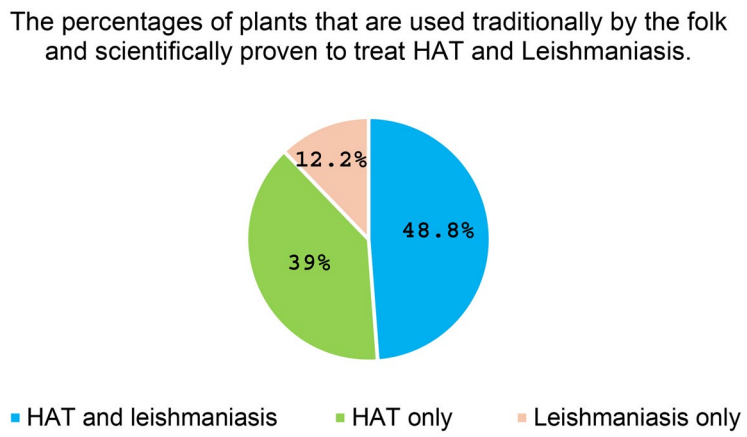

Figure 2. Percentage of plants traditionally used by local communities and scientifically validated for the treatment of HAT and leishmaniasis. (Figure self-drawn).

to treat HAT elicit robust antitrypanosomal activity, positioning them as promising candidates for potential drug development as alternative treatments for Human African Trypanosomiasis (HAT).

Furthermore, Table S1 provides an overview of traditionally used plants in treating leishmaniasis and HAT across various countries and regions. Notably, these diseases predominantly afflict tropical and subtropical regions. Therefore, most plants traditionally used to treat leishmaniasis and HAT are in those areas. The plants listed in Table S1 demonstrate robust antileishmanial and/or antitrypanosomal activity, characterized by an IC_{50} value of < 10 µg/mL. If there is no specific study addressing the antileishmanial or antitrypanosomal properties of plants in Table S1, we considered studies involving plants from the same genus that were scientifically proven to possess antiparasitic effects. This approach ensures a comprehensive summary of plants with strong antileishmanial and antitrypanosomal activities for a more focused and meaningful analysis.

Table 1 depicts about the plants that are traditionally used and proven to have antileishmanial and antitrypanosomal activities. Though some plants were not traditionally employed by local communities for treating leishmaniasis and HAT, they share the same genus as those traditionally used plants. Hence, these plants are also included in the table. Among the 41 traditionally used plants, almost half (20) have been scientifically validated for robust antitrypanosomal and antileishmanial activities against the parasites namely *Trypanosoma brucei* and *Leishmania*, demonstrating relatively moderate and low cytotoxicity (Table 1). However, an exception was observed with *Albizia zygia* (DC.) J.F. Macbr. Extract, which exhibited high cytotoxicity (<10 µg/mL) against L6 cells. Table 1 highlights that only *Achillea ptarmica* L., *Allium sativum* L., *A. cepa* L. and *A. ursinum* L. were reported to contain isolated compounds with antileishmanial and antitrypanosomal activities. Pellitorine, 8,9-*Z*-Dehydropellitorine, and (*E*, *E*)-2,4-undecadien-8,10-diynoic acid isobutylamide (DDI), an alkamide were isolated from the dichloromethane extract of *A. ptarmica* L., demonstrating potent activity against both parasites (Althaus et al. 2014). In contrast, the mixture of (*E*, *E*)-2,4-undecadien-8,10-diynoic acid pideridide and (*E*, *E*)-2,4-undecadien-8,10-diynoic acid phenethylamide, together with anacycline, selectively affected *T. brucei rhodesiense (Tbr)* (Althaus et al. 2014).

The phytochemical analysis of the *A. sativum* L. and *A. ursinum* L. extracts revealed sulfur containing compounds like allicin and ajoene (Krstin et al. 2018a, 2018b). In contrast, analysis of *A. cepa* L. extract analysis revealed the presence of zwiebelane (a sulfur-containing compound) (Krstin et al. 2018a). Notably, *A. sativum* L. exhibited nearly five-fold stronger antiparasitic activity against *T. brucei* compared to *A. cepa* L. The antiparasitic effect was attributed to sulfur compounds forming disulfide bonds with thiol groups (trypanothione and trypanothione reductase) in parasites, thereby inhibiting crucial enzymes in *Trypanosoma* and *Leishmania* parasites that are essential for maintaining redox balance and defence against reactive oxygen species in parasites (Krstin et al. 2018a). Moreover, the Table 1 also depicts about the selectivity index (SI) which was calculated as the ratio of CC_{50} (50% cytotoxic concentration in host cells) to IC_{50} (50% inhibitory concentration against parasites), providing a measure of compound selectivity. The SI is a critical parameter used to evaluate the

Table 1. *In vitro* traditionally used plants species scientifically proven to have both antileishmanial and antitrypanosomal activity on both *leishmania* and *trypanosoma*, respectively.

Scientific Name/family	Plant part	Extract	Parasite	Extracts/Fractions/ Compounds	IC_{50} (µg/mL)	CC_{50} (µg/mL)	SI	Reference
Achillea ptarmica L.[u] (Asteraceae)	A	DCM	*Tbr*	DCM	0.67	27.9[b]	41.64	Althaus et al. (2014)
				Pellitorine	5.35	45.5[b]	8.51	
				8,9-*Z*-Dehydropellitorine	2.00	16.5[b]	8.25	
				(*E*, *E*)-2,4-undecadien-8,10-diynoic acid isobutylamide	6.66	46.1[b]	6.93	
				(*E*, *E*)-2,4-undecadien-8,10-diynoic acid pideridide + (*E*, *E*)-2,4-undecadien-8,10-diynoic acid phenethylamide	3.50	43.4[b]	12.4	
				Anacycline	5.12	48.7[b]	9.51	
			Ld[a]	Pellitorine	5.96	45.5[b]	7.63	
				8,9-*Z*-Dehydropellitorine	5.01	16.5[b]	3.3	
				(*E*, *E*)-2,4-undecadien-8,10-diynoic acid isobutylamide	8.87	46.1[b]	5.2	
Achillea millefolium L.[u] (Asteraceae)	A (flowering)	DE	*Ld*[a]	DE	6.76	41.2[b]	6.1	Althaus et al. (2014)
Albizia lebbeck (L.) Benth.[u] (Fabaceae)	Fr, St	M	*Tbb*	M	8.1	32.0[g]	3.9	Al-Musayeib et al. (2012b)
	Rb	E	*Tbr*	E	9.0	n.d.	n.d	Kamanzi Atindehou et al. (2004)
Albizia schimperiana Oliv. (Leguminosae)	L	DCM	*Tbr*	DCM	7.2	225.6[i]	31.33	Nibret and Wink (2011)
Albizia zygia J.F.Macbr. (Fabaceae)	Sb	M	*Ld*[a]	M	3.5	4.5[b]	1.3	Ndjakou Lenta et al. (2007)
			Tbr	M	0.2		22.5	
Allanblackia monticola Mildbr. ex Staner. (Guttiferae)	Sb	M	*Ld*[a]	M	1.8	55.6[b]	30.9	Ndjakou Lenta et al. (2007)
			Tbr	M	7.2		7.7	
Allium cepa L.[u] (Amaryllidaceae)	Cl	DCM	*Tbb*	DCM	4.59	44.56[n]	9.7	Krstin et al. (2018a)
				Zwiebelane	n.d.	n.d.	n.d.	
Allium sativum L. (Amaryllidaceae)	Cl	DCM	*Tbb*	DCM	0.95	22.27[n]	23.4	
				Allicin	n.d.	n.d.	n.d.	
				Ajoene	n.d.	n.d.	n.d.	
Allium ursinum L.[u] (Amaryllidaceae)	Cl	DCM	*Tbb*	DCM	1.45	23.71[n]	16.4	Krstin et al. (2018b)
				Allicin	n.d.	n.d.	n.d.	
				Ajoene	n.d.	n.d.	n.d.	
Commiphora wightii (Arn.) Bhandari[u] (Burseraceae)	GR	M	*Tbb*	M	8.1	23.2[g]	2.9	Okba et al. (2018)
			Tbr	M	7.6		3.1	
			Li[a]	M	9.5		2.4	
Commiphora myrrha (Nees) Engl.[u] (Burseraceae)	GR	M	*Tbb*	M	8.1	>64[g]	>7.9	
			Tbr	M	2.2		29.1	
Harungana madagascariensis Lam. ex Poir. (Hypericaceae)	Sb	M	*Ld*[a]	M	1.6	28.1[b]	17.6	Ndjakou Lenta et al. (2007)
			Tbr	M	7.8		3.6	
	Sb	Lyp	*Tbb*	Lyp	8.64	20.06[b]	2.3	Musuyu Muganza et al. (2012)
Hymenocardia acida Tul. (Phyllanthaceae)	L	MC	*Tbb*	MC	5.0	12.2[b],	2.4	Hoet et al. (2004)
			Tbr	MC	9.1	28.0[c]	3.1	
Lantana ukambensis (Vatke) Verdc. (Verbenaceae)	St, L	M	*Ld*[p]	M	6.9	n.d.	n.d.	Sawadogo et al. (2012)
			Tbb	M	1.5	n.d.	n.d.	
Leucas cephalotes (Roth) Spreng. (Lamiaceae)	Wp	C	*Tbr*	C	5.15	31.1[b]	6.03	Dua et al. (2011)
			Ld[a]	C	3.61	31.1[b]	8.6	
Leucas inflata Benth.[u] (Lamiaceae)	L, Fl	M	*Tbb*	M	8.4	29.5[g]	3.5	Al-Musayeib et al. (2012a)
Nepeta nuda subsp. *Nuda* L.[u] (Lamiaceae)	A	M	*Tbr*	M	3.43	>90[b]	>26.2	Kirmizibekmez et al. (2011)
		H		H	0.62	85[b]	137.1	
		C		C	3.57	46.2[b]	13.0	
		H	*Ld*[a]	H	7.73	85[b]	11.0	
		C		C	4.08	46.2[b]	11.3	
Solanum villosum Mill.[u] (Solanaceae)	A	M	*Li*[a]	M	2.0	>64[g]	>32.0	Okba et al. (2018))
Solanum diphyllum L.[u] (Solanaceae)	Rt	M	*Tbb*	M	8.3	>64[g]	>7.7	

(*Continued*)

Table 1. Continued.

Scientific Name/family	Plant part	Extract	Parasite	Extracts/Fractions/ Compounds	IC_{50} (µg/ mL)	CC_{50} (µg/mL)	SI	Reference
Stereospermum zenkeri K.Schum. ex De Wild. (Bignoniaceae)	Sb	EA	*Ld*[a]	EA	3.7	>90[b]	>24.3	Ndjakou Lenta et al. (2007)
			Tbr		9.6		>9.4	

IC_{50}: concentration of compound or extract or fraction that inhibits 50% of parasite growth., CC_{50}: concentration of compound or extract or fraction that causes 50% cytotoxicity in host cells, SI: Selectivity Index ($SI = CC_{50}/IC_{50}$), EOCN: Fraction of Essential oil of *Cymbopogon nardus,* n.d. = not determined.

[a] = Amastigotes stage, [b] = Rat skeletal myoblast cell line (L6), [c] = Murine macrophage-like cell line (J774.A1), [d] = Fibroblast (NIH/3T3) cells, [e] = Chinese Hamster Ovary (CHO),), [f] = Peritoneal Macrophage, [g] = MRC-5 cells, [h] = mamalian cell line (Vero) (Monkey kidney), [i] = Human leukemia (HL-60) cells, [j] = Human non cancer fibroblast cell line (WI38), [k] = RAW 264.7 macrophages, [l] = HeLa (human cervix adenocarcinoma), [m] = BALB/3T3 mouse fibroblast cells, [n] = Immortalized human keratinocytes (HaCaT), [o] = MDCK cells, [p] = Promastigotes stage, [q] = KB cells, [r] = Transformed human monocytic (THP1) cells, [s] = BALB/c murine peritoneal macrophages, [t] = murine macrophages, [u] = Plants that does not used traditionally to treat leishmaniasis and HAT but proven to have antileishmanial and/or antitrypanosomal activities and have the same *genus* with plants that used traditionally to treat those diseases; *Tbb: Trypanosoma brucei brucei*, *Tbr: Trypanosoma brucei rhodesiense*, *Ld: Leishmania donovani, Li: Leishmania infantum*, *Lb: Leishmania braziliensis, Ltr: Leishmania tropica*; L: Leaves, St: Stems, B: Barks, Sb: Stem barks, Rb: Root barks, Rt: Roots, S: Seeds, Fl: Flowers, Fr: Fruits, Tw: Twigs, Wp: Whole plants, A: Aerial, Cl: Cloves; DCM: Dichloromethane, M: Methanol, C: Chloroform, EA: Ethyl acetate, E: Ethanol, H: Hexane, MC: Methylene chloride, DE: Diethyl ether, Lyp: Lyophilized.

therapeutic potential of bioactive compounds, as it reflects the degree to which a compound selectively targets parasites over host (mammalian) cells. A high SI value (>10) indicates that the compound is substantially more toxic to parasites than to host cells, making it desirable for drug development. Conversely, a low SI (<10) suggests poor selectivity, while an SI of 1 denotes equal toxicity to both parasite and host (Cos et al. 2006). For example, the methanol extract of *Cymbopogon citratus* (DC.) Stapf with an IC_{50} of 4.4 µg/mL against *Trypanosoma brucei* and a CC_{50} greater than 100 µg/mL in mammalian cells, yielding an SI of >22.5 (Table 2). This demonstrates good selectivity and highlights its potential as a promising lead compound.

Furthermore, Table 2 illustrates the plants that are traditionally used to treat both diseases; HAT and leishmaniasis which were scientifically proven to manifest either strong antitrypanosomal or antileishmanial activity against *Trypanosoma brucei* or *Leishmania* parasites, respectively. A strong antitrypanosomal effect was observed with 16 out of the 41 plants examined, while only five traditionally utilized plants demonstrated potent antileishmanial activity. This emphasized a prevalence of strong antitrypanosomal effects compared to potent antileishmanial activity among the assessed plants. Noteworthy findings from Table 2 were that the *Ficus* and *Cymbopogon* genus were the majority of plants which exhibited antitrypanosomal activity toward *Trypanosoma brucei*, with each contributing four species. However, *Cymbopogon* species outshined *Ficus* species regarding antitrypanosomal activity, with *Cymbopogon giganteus* Chiov. exhibiting the most potent effect at < 0.25 µg/mL (Kpoviessi et al. 2014). On the other hand, *Viola canescens* Wall. ex Roxb. showcases the most potent antileishmanial activity (0.4 µg/mL) against *Leishmania donovani (Ld)* (Dua et al. 2011). Additionally, only seven plants namely *Strychnos spinosa* Lam., *Cymbopogon nardus* (L.) Rendle, *C. citratus* (DC.) Stapf, *C. giganteus* Chiov., *C. schoenantus* (L.) Spreng., *Keetia leucantha* (K.Krause) Bridson and *Tridax procumbens* L. were reported on their active compounds that exhibited strong antitrypanosomal effects. The compounds isolated from *Strychnos spinosa* Lam. included (*E*)-Nerolidol, linalool, (+)-terpinen-4-ol from its essential oil and erythrodiol, botulin, ursolic acid, oleanolic acid, saringosterol and 24-hydroperoxy-24-vinylcholesterol from its dichloromethane extract (Hoet et al. 2006; Hoet et al. 2007). Nerolidol (*E*- and *Z*-mixture) have been demonstrated to block an unspecified but initial stage in the mevalonate pathway, thereby inhibiting the biosynthesis of isoprenoids (e.g., dolichol, ergosterol, and ubiquinone) in *L. amazonensis* (Hoet et al. 2006). Apart from this, oleanolic and ursolic acids have demonstrated the ability to induce apoptosis and inhibit various enzymes, including human topoisomerases I and II (Hoet et al. 2007). The carboxylic acid functional group in these compounds appears crucial for their remarkable inhibitory effects on these DNA-manipulating enzymes (Hoet et al. 2007). Nonetheless, the specific mechanisms of their actions remain unclear and yet to be explored meticulously. On the other hand, essential oils from four *Cymbopogon* species contained myrcene, *R*-(+)-limonene, citral, citronellal, and β-citronellol (Kpoviessi et al. 2014). At the same time, *K. leucantha*'s dichloromethane extract yielded ursolic and oleanolic acids (Bero et al. 2011). Conversely, the isolated compound from the methanolic extract of *T. procumbens* L., namely oxylipin

Table 2. *In vitro* traditionally used plants species scientifically proven to have either strong antitrypanosomal activity against *trypanosoma* parasite or strong antileishmanial activity against *leishmania* parasite.

Scientific Name / family	Plant part	Extract	Parasite	Extracts/Fractions/ Compounds	IC_{50} (µg/mL)	CC_{50} (µg/mL)	SI	Reference
***Trypanosoma brucei* parasites**								
Artemisia roxburghiana Besser (Asteraceae)	L	PE	*Tbr*	PE	6.0	23.7[b]	3.95	Dua et al. (2011)
Cymbopogon citratus (DC.) Stapf (Poaceae)	Rh	M	*Tbb*	M	4.44	>100[h]	>22.5	Norhayati et al. (2018)
		EO		EO	1.83	10.63[e], 39.77[j]	5.8, 21.73	Kpoviessi et al. (2014)
Cymbopogon giganteus Chiov. (Poaceae)	Wp	EO	*Tbb*	EO	0.25	>50[e], >50[j]	>200, >200	Kpoviessi et al. (2014)
Cymbopogon nardus (L.) Rendle (Poaceae)	Wp	M	*Tbb*	M	0.31	> 100[h]	>322.58	Norhayati et al. (2018)
		EO		EO	5.71	>50[e,] >50[j]	>8.76, >8.76	Kpoviessi et al. (2014)
		EO and its fractions		EOCN1	0.31	>100[h]	>322.58	Muhd Haffiz et al. (2013)
				EOCN2	3.94	>100[h]	>25.38	
				EOCN3	0.44	>100[h]	>227.27	
				EOCN4	0.30	>100[h]	>333.33	
				EOCN5	0.59	>100[h]	>169.49	
				EOCN6	1.46	>100[h]	>68.49	
				EOCN7	3.26	>100[h]	>30.67	
				Subfractions F4	0.61	77.41[h]	126.90	
				Subfractions F6	0.73	>100[h]	>136.99	
				Subfractions F7	1.15	>100[h]	>90.09	
				Subfractions F8	1.11	>100[h]	>90.09	
Cymbopogon schoenantus (L.) Spreng. (Poaceae)	L	EO	*Tbb*	EO	2.10	>50[e], >50[j]	>23.8, >23.8	Kpoviessi et al. (2014)
				*Myrcene	2.24	>50[e], >50[j]	>22.32, >22.32	
				**R* (+)-limonene	4.24	>50[e], >50[j]	>11.79, >11.79	
				*Citral	5.98	20.62[e], 39.48[j]	3.45, 4.93	
				*Citronellal	2.76	>50[e], >50[j]	>18.12, >18.12	
				*β-Citronellol	6.45	>50[e], >50[j]	>7.75, >7.75	
Dovyalis abyssinica (A. Rich.) Warb. (Salicaceae)	L	M	*Tbb*	M	2.9	167.2[i]	57.66	Nibret and Wink (2011)
		DCM		DCM	1.4	174.9[j]	124.93	
Ficus cordata ssp. *Salicifolia* (Vahl ex Hook. f.) C.C. Berg (Moraceae)	L, St	M	*Tbb*	M	8.2	32.5[g]	3.96	Al-Musayeib et al. (2012a)
Ficus elastica Roxb. ex Hornem. (Moraceae)	AR	M	*Tbb*	M	0.9	20.9[i]	23.22	Mbosso Teinkela et al. (2018)
Ficus ingens (Miq.) Miq. (Moraceae)	L, St	M	*Tbb*	M	8.0	32.5[g]	4.06	Al-Musayeib et al. (2012a)
Ficus palmata Forssk. (Moraceae)	L, St	M	*Tbb*	M	8.1	37.7[g]	4.65	
Holarrhena africana A.DC. (Apocynaceae)	L	Aq	*Tbb*	HaF4	4.5	n.t.	n.d.	Nwodo et al. (2007)
				HaF5	0.785	26.3[b]	33.50	
Keetia leucantha (K.Krause) Bridson (Rubiaceae)	Tw	DCM	*Tbb*	DCM	5.8	>100[j]	17.24	Bero et al. (2011)
				Oleanolic acid	2.9	n.d.	n.d.	
				Ursolic acid	1.1	n.d.	n.d.	
Mentha piperita L. (Lamiaceae)	L	C	*Tbr*	C	5.46	60.55[b]	11.09	Dua et al. (2011)
Ozoroa insignis Delile (Anacardiaceae)	St, L	M	*Tbb*	M	6.2	n.t.	n.d.	Sawadogo et al. (2012)

(*Continued*)

Table 2. Continued.

Scientific Name / family	Plant part	Extract	Parasite	Extracts/Fractions/ Compounds	IC_{50} (µg/mL)	CC_{50} (µg/mL)	SI	Reference
Strychnos spinosa Lam. (Loganiaceae)	L	MC	*Tbb*	MC	1.5	25.9[b],100[c]	17.27, 66.67	Hoet et al. (2004)
		EO		(*E*)-Nerolidol	1.7	>100[c]	>58.82	Hoet et al. (2006)
				Linalool	2.5		40	
				(+)-terpinen-4-ol	6.5		15.38	
		DCM		Erythrodiol	5.3	22.3[c]	4.21	Hoet et al. (2007)
				Betulin	4.0	29.7[c]	7.43	
				Ursolic acid	1.0	6.0[c]	6	
				Oleanolic acid	2.9	59.6[c]	20.55	
				Saringosterol	3.3	>100[c]	>30.30	
				24-hydroperoxy-24-vinylcholesterol	1.4	7.3[c]	5.21	
Vitellaria paradoxa C.F. Gaertn. (Sapotaceae)	B	EA	*Tbb*	EA	3.25	n.t.	n.d.	Bairy et al. (2023)
***Leishmania* parasites**								
Piper angustifolium Lam. (Piperaceae)	L	EO	*Li*[a]	EO	1.43	48.22[d],31.67[c]	33.72, 22.15	Bosquiroli et al. (2015)
Tridax procumbens L. (Asteraceae)	Wp	M	*Lm*[p]	M	3.0	>100[b]	>33.33	Martín-Quintal et al. (2009)
				Oxylipin (3*S*)-16,17-didehydrofalcarinol	0.478	47.8[b]	~ 100	
	A	DCM	*Ld*[a]	DCM	4.0	43.78[b]	10.95	Nour et al. (2009)
Viola canescens Wall. ex Roxb. (Violaceae)	Wp	PE	*Ld*[a]	PE	0.4	12.4[b]	30	Dua et al. (2011)

*Contain in the essential oil of all four *Cymbopogon species* study in Kpoviessi et al. (2014). n.t.: not tested, n.d.= not determined, IC_{50}: concentration of compound or extract or fraction that inhibits 50% of parasite growth, CC_{50}: concentration of compound or extract or fraction that causes 50% cytotoxicity in host cells, SI: Selectivity Index ($SI = CC_{50}/IC_{50}$), HAF: *Holarrhena Africana* Fraction, EOCN: Essential oil *Cymbopogon nardus*; [a] = Amastigotes stage, [b] = Rat skeletal myoblast cell line (L6), [c] = Murine macrophage-like cell line (J774.A1), [d] = Fibroblast (NIH/3T3) cells, [e] = Chinese Hamster Ovary (CHO),), [f] = Peritoneal Macrophage, [g] = MRC-5 cells, [h] = mammalian cell line (Vero) (Monkey kidney), [i] = Human leukemia (HL-60) cells, [j] = Human non cancer fibroblast cell line (WI38), [k] = RAW 264.7 macrophages, [l] = HeLa (human cervix adenocarcinoma), [m] = BALB/3T3 mouse fibroblast cells, [n] = Immortalized human keratinocytes (HaCaT), [o] = MDCK cells, [p] = Promastigotes stage, [q] = KB cells, [r] = Transformed human monocytic (THP1) cells, [s] = BALB/c murine peritoneal macrophages, [t] = murine macrophages; *Tbb: Trypanosoma brucei brucei*, *Tbr: Trypanosoma brucei rhodesiense*, *Ld: Leishmania donovani*, *Li: Leishmania infantum*, *Lm: Leishmania mexicana*; L: Leaves, St: Stems, B: Barks, Sb: Stem barks, Rb: Root barks, Rt: Roots, S: Seeds, Fl: Flowers, Fr: Fruits, Tw: Twigs, Wp: Whole plants, A: Aerial, Rh: Rhizomes; DCM: Dichloromethane, M: Methanol, C: Chloroform, EA: Ethyl acetate, E: Ethanol, Aq: Aqueous, EO: Essential oils, MC: Methylene chloride, PE: Petroleum ether.

(3*S*)-16,17-didehydrofalcarinol (Figure 3), displayed the highest antileishmanial activity against *Leishmania mexicana* (0.478 µg/mL) compared to other compounds (Table 2) (Martín-Quintal et al. 2009). Notably, all plant extracts and active compounds in Table 2 showed potent antitrypanosomal or antileishmanial effects with minimal cytotoxicity, except for the ursolic acid and 24-hydroperoxy-2 4-vinylcholesterol from the dichloromethane extract of *S. spinosa* Lam.

In addition, Table S2 delineates plants which are not traditionally employed for treating leishmaniasis and trypanosomiasis yet exhibited both antileishmanial and antitrypanosomal effects against *Leishmania* and *Trypanosoma* parasites. A total of 47 plants demonstrates antiparasitic efficacy, prominently featuring the genus *Salvia*, including *S. spathacea* Greene, *S. tomentosa* Mill., *S. sclarea* L. and *S. dichroantha* Stapf, with generally moderate and low cytotoxicity, except for the hexane extract of *S. sclarea* L. (18.3 µg/mL) (Kirmizibekmez et al. 2011). Within this classification, several plants demonstrated singular effects. However, if a plant exhibited solely antitrypanosomal effects and another plant from the same genus displayed antileishmanial effects, they have been put together in Table S2. This is because there was a high possibility that other plants within the same genus may exert antiparasitic effects against both parasites. Further investigations into plants from the same genus is required for a comprehensive understanding about their antiparasitic potential. In Table S2, only *Marrubium incanum* Desr., *Selaginella sellowii* Hieron., *Xanthium brasilicum* Vell., *Anacyclus pyrethrum* (L.) Lag. and *Tulbaghia violacea* Harv. had reported isolated compounds. The isolated compound from *M. incanum* Desr. known as salvigenin, which only exhibited the prominent antitrypanosomal effects. Salvigenin contains a phenolic hydroxyl group at C-5, which was found to be responsible for its ability to readily dissociate, forming a negatively charged phenolate ion under physiological conditions. This ion efficiently interacts with various proteins, including enzymes, transcription factors and carriers through

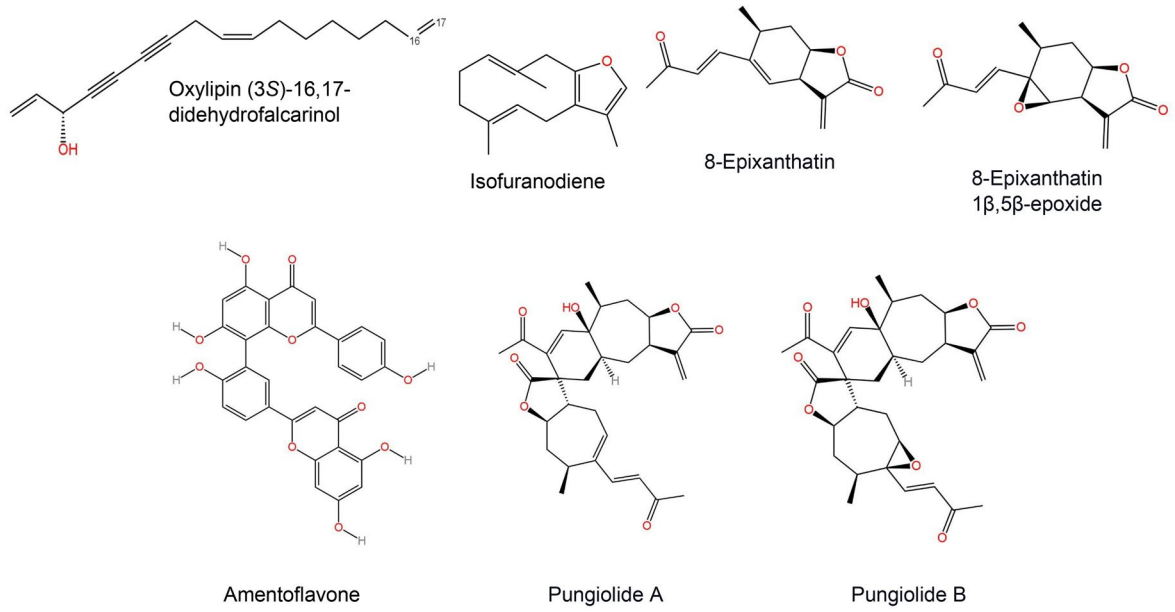

Figure 3. Chemical structures of isolated compounds with IC_{50} values < 1 µg/mL. All structures were generated using ChemDraw.

ionic bonding or hydrogen bonds, which lead to a conformational change in the target proteins, subsequently altering their biological activities. Along with this, salvigenin; which is also a flavonoid, possesses a distinctive capability to modulate gene expression, potentially influencing crucial genes involved in preventing viral and parasitic infections (Frezza et al. 2020). The passage hypothesizes that these mechanisms of action might contribute to the observed antitrypanosomal activity of salvigenin. Further investigations are needed to elucidate the precise mechanism of action of salvigenin.

Apart from this, *S. sellowii* Hieron. yielded biflavonoids namely amentoflavone (Figure 3) and robustaflavone (Figure S5), demonstrating significant antileishmanial activity against *Leishmania*, while the isolated compounds from *X. brasilicum* Vell., namely 8-epixanthatin, 8-epixanthatin 1β,5β-epoxide, pungiolide A, pungiolide B, and 4,15-Dinor-1,11(13)-xanthadiene-3,5β:12,8β-diolide exhibited strong antileishmanial and antitrypanosomal activities (Nour et al. 2009; Rizk et al. 2014). Furthermore, the *A. pyrethrum* (L.) Lag. yielded several active compounds, including pellitorine, 9,10-dehydropellitorine, deca-2*E*,4*E*-dienoic acid 2-phenylethylamide, undeca-2*E*,4*E*-dien-8,10-diynoic acid isopentylamide, tetradeca-2*E*,4*E*,12*Z*-trien-8,10-diynoic acid isobutylamide, dodeca-2*E*,4*E*-dienoic acid 4-hydroxy-2-phenylethylamide, undeca-2*E*,4*E*-dien-8,10-diynoic acid 2-phenethylamide, and deca-2*E*,4*E*-dienoic acid 4-hydroxy-2-phenylethylamide (Althaus et al. 2017). These compounds affected both *Leishmania* and *Trypanosoma* parasites, while anacycline exclusively affected *Trypanosoma* parasite. However, despite their bioactivity, all these compounds except salvigenin, anacycline, pellitorine, 9,10-dehydropellitorine, undeca-2*E*,4*E*-dien-8,10-diynoic acid isopentylamide and tetradeca-2*E*,4*E*,12*Z*-trien-8,10-diynoic acid isobutylamide displayed high cytotoxicity. Besides, the main compound isolated from *T. violacea* Harv. extract was marasmicin (allicin analogue) (Krstin et al. 2018b). The action of this compound was the same as that of *Allium* species, involving sulfur compounds forming disulfide bonds with free thiol groups inside parasites, thereby inactivating vital substances for their survival (Krstin et al. 2018b). In Table S2, the majority of plants exhibited moderate to low cytotoxicity, except for the extracts of *S. sellowii* Hieron., *Francoeuria crispa* (Forssk.) Cass., *Xanthium strumarium* L., *X. brasilicum* Vell., *Piptadeniastrum africanum* (Hook.f.) Brenan, *Pyrenacantha grandiflora* Baill. and root bark extract of *Quassia africana* Baill. with *P. africanum* (Hook.f.) Brenan methanolic extract being the most toxic among them (<0.25 µg/mL). These findings revealed the potential of traditional medicinal plants to treat parasitic diseases by providing novel compounds with antileishmanial and antitrypanosomal activities for further exploration in drug development against these parasitic diseases.

Moreover, Table S3 unveils a comprehensive list of 25 plants and 104 plants that were not traditionally employed for leishmaniasis and HAT treatment, respectively showcases potent antileishmanial activity and robust antitrypanosomal activity against *Leishmania* and *Trypanosoma brucei* parasites, respectively. Among the plants exhibiting antileishmanial activity, only extracts from *Antrocaryon klaineanum* Pierre, *Khaya senegalensis* (Desr.) A. Juss., *Berberis vulgaris* L. and *Mimosa caesalpiniifolia* Benth. have been reported with their active compounds. Noteworthy compounds isolated from *Antrocaryon klaineanum* Mart. include antroklaicerebroside, scopoletin, 3,3′-Dimethylellagic acid, β-sitosterol, and stigmasterol (Amang à Ngnoung et al. 2023b). Scopoletin (coumarin) was suggested to act by damaging the mitochondrial membrane, causing ultrastructural changes in the *Leishmania* parasite (Amang à Ngnoung et al. 2023b). Additionally, *K. senegalensis* (Desr.) A. Juss. contained active compounds such as bellericagenin B, gynuramide IV, β-sitosterol glycoside, and stigmasterol glycoside (Amang à Ngnoung et al. 2023a). Interestingly, the antiparasitic activity of β-sitosterol glycoside and stigmasterol glycoside was tested in combination. On the other hand, berberine was an active compound from *B. vulgaris* L., which claimed to exhibit antileishmanial activity. However, in the study, the compound was not isolated from the plant extract but obtained from Sigma-Aldrich (Mahmoudvand et al. 2014). Furthermore, betulinic acid was isolated from *M. caesalpiniifolia* Benth. extract as an active compound (Brito et al. 2021). Besides this, the hexane extract of *Dipteryx alata* Vogel displayed the most potent antileishmanial activity with an IC_{50} value of 0.08 µg/mL with moderate toxicity toward mammalian cells. This extract rich in phenolic compounds such as tannins, holds the potential to play a crucial role in its antileishmanial activity. However, further research is necessary to confirm this claim (Ribeiro et al. 2014). Importantly, all of the plant extracts and bioactive compounds exhibit no significant cytotoxicity, emphasizing their potential safety for therapeutic applications. The only exceptions were *Saussurea costus* (Falc.) Lipsch., *Ximenia caffra* Sond. extracts and the hexane extract of *Casearia sylvestris* (Cambess.) Eichler root bark part. This collective evidence highlights the pharmacological richness of the traditional medicinal plants, offering promising avenues for developing safe and effective treatments against leishmaniasis.

Furthermore, the *Combretum* genus was found to be predominant among the plants exhibiting significant antitrypanosomal activity against *Trypanosoma brucei* parasite, with contributions from four plant species: *C. molle* R.Br. ex G.Don, *C. racemosum* P.Beauv., *C. smeathmannii* G.Don and *C. hartmannianum* Schweinf. that exhibited a strong effect on *Tbr* with low cytotoxicity. Intriguingly, only two plant species in this category namely *Amaryllis belladonna* L. and the essential oil of *Smyrnium olusatrum* L. have been reported with the isolated compounds demonstrating antitrypanosomal activity against *Trypanosoma brucei* parasite. *A. belladonna* L. yielded 1-O-acetylcaranine and 3-O-acetylhamayne (Figure S5) (Tallini et al. 2017; Frezza et al. 2020). However, 3-O-acetylhamayne displayed high cytotoxicity (1.72 µg/mL) on fibroblast (NIH/3T3) cells. Besides this, *Smyrnium olusatrum* L. essential oil contains isofuranodiene as an active compound (Petrelli et al. 2017). Isofuranodiene, being highly hydrophobic, may be easily absorbed by a cell membrane, thereby inducing destabilization of the phospholipid bilayer (Petrelli et al. 2017). Notably, it can also modify the permeability of both outer and inner mitochondrial membranes in eukaryotic cells, resulting in apoptotic effects (Petrelli et al. 2017). The cytotoxicity profiles of most plant extracts in this category are generally moderate to low, with the exception of *Kanahia laniflora* (Forssk.) R.Br., *Kniphofia sumarae* Schweinf. ex Engl., *Momordica charantia* L., *Omphalocarpum glomerate* (P.Beauv.), *Connarus suberosus* Planch., *Enantia chlorantha* Oliv., *Trichilia emetica* Vahl, and *Trichilia monadelpha* (Thonn.) J.J.De Wilde. Overall, these findings highlight the promise of traditional medicinal plants as valuable sources of novel antitrypanosomal compounds. Their potential warrants further investigation, particularly for the development of safe and effective therapies targeting neglected tropical diseases, including New World leishmaniasis.

Conclusions

This comprehensive review highlights the wealth of plant resources with potent antileishmanial and antitrypanosomal activities, emphasizing their potential as alternative treatments for leishmaniasis and HAT. The most traditional plants that exhibit strong antileishmanial and antitrypanosomal effects

were not initially utilized for treating leishmaniasis and HAT. Nevertheless, they demonstrate significant potential as safe and cost-effective alternatives to conventional medications. Importantly, there remains a critical therapeutic gap in addressing New World leishmaniasis, where current treatment options are scarce and often inadequate, underscoring the urgent need to explore plant-derived compounds as potential safe and effective alternatives. However, further research, including a study on the isolation of the bioactive compounds from the plants, clarification of their mode of action, and a comprehensive assessment of these compounds' efficacy and toxicity profiles, needs to be carried out before confirming their antiparasitic effect. At the same time, *in vivo* studies are essential to validate their efficacy and safety for potential drug development as an antiparasitic agent.

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Data availability statement

Data sharing is not applicable to this article as no data were created or analyzed in this study. We also state that a review protocol was not prepared.

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