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Antileishmanial Activity of the Genus *Piper*: A Systematic Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Systematic Review

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ABSTRACT

Background: Leishmaniases are anthropozoonotic vector-borne diseases, caused by the protozoan *Leishmania* spp. These diseases have significant morbidity and mortality worldwide and, as there are currently no vaccines available for their treatment, chemotherapy remains the mainstay for anti-leishmanial therapeutics. However, the severe side effects, reduced bioavailability, high cost and chemoresistance, amongst other problems, limit the use of available drugs. In recent years, natural compounds have shown promise as anti-leishmanial agents, especially those extracted from medicinal plants. The genus *Piper* has been used in traditional medicine and widely explored for its biological properties and bioactive phytocompounds.
Aim: The aim of this study was to conduct a systematic literature review of the biological activity of

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the genus *Piper* against the etiological agents of leishmaniasis, to provide a perspective for effective and safe phytotherapics, new drugs or potentially active prototype chemical substances.
Methodology: This systematic review was prepared in accordance with PRISMA guidelines. The databases used for this review were SciELO, Pubmed, ScienceDirect and Google Scholar, using a temporal profile of 2009 to 2020.
Conclusion: In this review, we summarize a wide range of isolated compounds, extracts and essential oils of the genus *Piper* that are worth screening given their potential for development as effective anti-leishmanial drugs.

Keywords: Leishmaniasis; Leishmania spp.; Piperaceae; Piper; Phytotherapics; Natural products.

1. INTRODUCTION

Leishmaniases are parasitic diseases that are caused by over 20 species of the protozoan genus *Leishmania* and infected vector-borne. According to the World Health Organization [1], leishmaniasis constitutes a serious problem in public health and is endemic in 98 countries, affecting 12 million people globally. The disease is considered to be a neglected tropical disease that mostly affects economically disadvantaged individuals in many developing countries. The infection can lead to different clinical manifestations, including visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (ML) [1]. VL, also known as kala-azar, is fatal if left untreated, has a systemic character, and mainly affects the spleen and liver. The tegumentary forms have a major impact on patients' quality of life because the disease can lead to deformities and social stigma [2]; CL, the most common form, causes skin lesions, mainly ulcers, on exposed parts of the body; and ML, causes devastating partial or total destruction of the mucous membranes of the nose, mouth and throat [1].

Leishmaniasis is endemic in Brazil; In 2019, of the total number of VL cases in the Americas, 97% were from Brazil. Additionally, cases of co-infection of CL-HIV and VL-HIV have increased over the years, in Brazil, as well as the mortality rate of cases of VL [1]. Even with the high incidence of leishmaniasis in Brazil and worldwide, there exist few alternatives for its treatment and the therapeutic agents currently available are inefficient.

The high incidence of leishmaniasis, associated with serious treatment limitations in terms of safety, resistance, stability and cost, low tolerability, long duration, and difficult administration, hinder its treatment [3,4]. The first-line drugs that are most used for the treatment of leishmaniasis are the pentavalent antimonials: meglumine antimoniate and sodium stibogluconate. Second-line drugs include amphotericin B, pentamidine, miltefosine and paromomycin [3,5]. The number of drugs available is still limited and none has a totally satisfactory result, since they are also highly hepatotoxic, nephrotoxic and cardiotoxic, resulting in poor adherence to treatment, and compromising the effectiveness of therapy [4,5]. Given this scenario, studies are needed that aim to identify more efficient and less toxic active compounds. Within this context, plants are an important source of natural bioactive products, from which many drugs have been derived and marketed worldwide. The chemical and pharmacological study of medicinal plants has made it possible to obtain new compounds with different properties [6].

The Amazon region has a rich biodiversity that includes a large number of plant species that are commonly used in traditional medicine and have contributed to the discovery of several bioactive species [7]. Studies have increased and greater emphasis has been placed on Amazonian species of phytochemical interest [7,8]. Among these species with pharmacological potential, those of the genus *Piper* stand out, due to their use in traditional medicine and promising biological activities [8,9].

Piper is the largest genus of the family Piperaceae, with nearly 2000 species of herbs, shrubs, or small trees distributed throughout the tropical and subtropical forests, primarily in Central and South America, the Caribbean, Africa, Asia, and the Pacific Islands [10]. In fact, these plants have been commonly used in traditional medicine [8,11–12] and are an important source of therapeutic agents [13], as they display a great chemical diversity of secondary metabolism [10,14], including alkaloids [15], flavonoids [16], terpenoids [17], phenylpropanoids [18], phenolic acids [19] and lignans [20]. These constituents have been

reported to have antioxidant [15], anti-inflammatory [21], antitumor [22], and antiparasitic [19,20] properties.

Thus, this review aims to report on the biological activity of the genus *Piper* against the etiological agents of leishmaniasis, from the perspective of the search for effective and safe phytotherapics, new drugs or prototypes of potentially active chemical substances.

2. METHODS

2.1 Search Strategy

This is a systematic literature review study based on the recommendations proposed by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyzes) guide [23]. Consultations were performed on the databases, SciELO (Scientific Electronic Library Online), Pubmed (US National Library of Medicine), ScienceDirect and Google Scholar, from May 2019 to February 2020. The temporal profile of articles consulted was from January 2009 to January from 2020. The following search keywords were used, "*Leishmanicidal Piper"; "Antileishmanial Piper"; "Leishmania Piper"; "Piper leishmaniasis"; "Leishmanicidal activity Piper*".

2.2 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria of the articles in this review have been described previously [24]. In brief, only the following articles were included: Those from original journals, in English, Portuguese and Spanish, articles that presented a half maximal inhibitory concentrations (IC_{50}), those that address some species of the use of *Piper* genus against *Leishmania* (where scientific names were validated through The Plant List; www.theplantlist.org), and experimental design studies using oil, extract, fraction or isolates of *Piper* species compounds in *in vivo* or *in vitro* assays. The flowchart shows the selection of articles, as well as the inclusion and exclusion of studies (Fig. 1). For data extraction, a tool was elaborated containing: Scientific name, Plant part, Country, Compound / Extract, *Leishmania* species, Results IC_{50} of leishmanicidal activity and references (Table 1).

3. ANTILEISHMANIAL ACTIVITY OF THE GENUS *Piper*

3.1 Study Inclusion

The systematic review included four steps (Fig. 1): (i) Identification (selection of databases and preliminary survey of articles, identifying 173 articles, excluding 67 duplicates); (ii) Selection (15 were excluded after temporal clipping for 2009-2020 and 40 other articles were excluded after analyzing the titles and abstracts); (iii) Eligibility (51 Studies that addressed the genus *Piper* and Leishmaniasis were selected for full reading and 11 studies were excluded for not meeting inclusion criteria); and (iv) Inclusion (the Systematic Review was conducted with 40 final articles).

3.2 Result Tables

(Table 1) describes the main results of the selected articles; 46 different species of the genus *Piper* were used in the studies. Among the species investigated, *Piper aduncum* was the most studied and majority of the studies were from Brazil and Colombia. The leishmanicidal activities of various plant extracts and isolated compounds were evaluated against promastigote and amastigote stages, with prevalence for the *Leishmania (Leishmania) amazonensis* species.

3.3 Essential Oils

Essential oils are highly volatile hydrophobic liquids that are obtained from different parts of plants, such as the flowers, leaves, bark, stems, wood, roots, fruits or seeds, and have been used in folk medicine to treat various diseases. The substances that are most abundant in these mixtures are terpenoids, especially mono and sesquiterpenes [65]. The major chemical constituents in the essential oils from the genus *Piper* are phenylpropanoids and terpenes [9].

In the search for alternative treatments for leishmaniasis, essential oils of 23 species of the *Piper* genus have been evaluated for toxicity towards promastigotes, amastigotes and mammalian cells (Table 1). Based on the IC_{50} values, the best results were obtained in essential oils of *Piper marginatum*, containing 3,4-methylenedioxypropiophenone (22.9%) and δ-3-carene (10.1%), and *Piper angustifolium*, containing spathulenol (23.8%) and caryophyllene oxide (13.1%); these were highly active against *L. (L.) amazonensis* (IC_{50} of 0.6 µg / mL) and *Leishmania infantum* (IC_{50} of 1.43 µg / mL) amastigotes, respectively. Both essential oils showed low toxicity to macrophages, *P. marginatum* (CC_{50} 34.5 µg / mL) and *P. angustifolium* (CC_{50} of 48.22 µg / mL) were more selective for parasites than for mammalian cells, as indicated by selectivity indices (SI of 57 and 33, respectively) [54; 33].

Another interesting essential oil is that of *Piper hispidum*, which is rich in sesquiterpenes, notably curzerene (15.7%); this oil was found to be active against the axenic amastigotes (IC_{50} 3.4 µg / mL) and peritoneal macrophages infected *with L. (L.) amazonensis* (IC_{50} 4.7 µg / mL) [50]. *In vitro* intracellular assays using primary cells, such as mouse peritoneal macrophages, are the most accurate models for providing reproducible results and a rate high of infection, which are essential for an accurate analysis, providing more relevant information on compound efficacy [66].

Other essential oil sources that have been found to be active against *L. (L.) amazonensis* and *Leishmania donovani*, are leaves of *Piper aduncum* (IC_{50} 36.2 µg / mL) and aerial parts of *Piper auritum* (IC_{50} 22.3 µg / mL), respectively. Both essential oils showed low toxicity against BALB/c mouse peritoneal macrophages, *P. aduncum* (CC_{50} >200 µg / mL) and *P. auritum* (CC_{50} of 106.4 µg / mL) [27;34]. Studies with the essential oil of leaves of *Piper demeraranum* and *Piper duckei* have identified values of IC_{50} of 78 and 42.4 µg / mL, respectively, against amastigote forms of *L. (L.) amazonensis*. The GC-MS analysis of *P. duckei* oil identified its main active chemical component as sesquiterpene trans-caryophylenne (27.1%) [46]. Bernuci et al. [27], when analyzing different species of the genus *Piper*, found that these species presented major concentrations of sesquiterpene compounds that contributed to their leishmanicidal activity. However, additional studies are required to explain the mechanism of action of these molecules on the amastigote form of the parasite.

Different mechanisms can explain the reported effects of essential oils on parasites, which include the induction of cell death by apoptosis and / or necrosis, interruption of the cell cycle and loss of function of the main organelles. Essential oils are small hydrophobic molecules that easily penetrate cell membranes and, consequently, gain access to intracellular targets, causing changes in the integrity of cell structures and the mitochondrial membrane [67-69]. However, almost all studies have stopped short of further investigating the leishmanicidal effects of essential oils of *Piper* and none have explored their *in vivo* effects. Only one study investigating the essential oil of *Piper* studied its additional leishmanicidal effects. The popular use of *P. demeraranum* and *P. duckei* for the treatment of skin wounds has led to new trials to assay its potential as a topical medicine. The HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) assay, a rapid and inexpensive assay, was used to show the irritation potential of these essential oils, demonstrating that these oils had a promisingly low level of irritability and did not cause bleeding or clotting of the membrane [46]. Findings indicate the potential for the utilization of these oils as auxiliary medications in cases of cutaneous leishmaniasis, as they reduce the adverse effects of drugs commonly used in the treatment of tegumentary leishmaniasis and show good penetration and permeability in the skin.

3.4 Crude Extracts and Fractions

Plant extracts are complex mixtures containing a variety of chemical constituents, known as secondary metabolites, with many functional groups in their structures. These functional groups on molecules can be active, partially active, and inactive and chemical interactions usually occur between these metabolites. Therefore, a high activity of a crude extract can result from the presence of many weakly active compounds that act in additive or synergistic manners [70].

In addition, the extraction method can strongly influence the chemical composition and, consequently, the biological activity of the extract. The process of separating different bioactive constituents from natural extracts consists of three main phases: extraction from plant matter, fractionation of the extract or oil, and purification of the active principle [71]. The activities of extracts and fractions of 20 species of the genus *Piper*, against different species of *Leishmania*, were evaluated in the studies reviewed (Table 1). Most studies looked at their effects on *Leishmania* species that cause tegumentary Leishmaniasis, such as *Leishmania (Viannia) panamensis, Leishmania (Viannia) guyanensis, Leishmania (V.) braziliensis* and *L. (L.) amazonensis*. Only four of the studies used *L. donovani*, which is the etiological agent of Old World visceral leishmaniasis. Most of the studies showed that the anti-leishmania properties used ethanol solvent for extraction and the aerial parts and roots of the genus *Piper*. The species *Piper amalago*, *Piper betle*, *Piper crassinervium, Piper dennisii, P. hispidum, P. marginatum, Piper nigrum, Piper ovatum, Piper sanguineispicum* and *Piper umbellatum* have all been reported to be promising anti-leishmanial agents [31; 38; 42,48,54,55,61]. The initial crude extracts are

often prepared with methanol or ethanol as solvents, which have been shown to extract a wide range of bioactive molecules [70].

Among the species studied, *P. marginatum* stands out; this species is commonly referred to as capeba, malvarisco, bush pepper or nhandi. Ethanolic extracts and different fractions of *P. marginatum* leaves presented anti-promastigote and anti-amastigote activity against *L. (L.) amazonensis* with low toxicity for macrophages. The ethanolic extract showed the best selectivity index in comparison to the reference drug, Pentamidine, with an SI of 415.8 and IC_{50} of 1.2 µg / mL, being considered a promising species against leishmaniasis [54]. Another study reported that hexanic and ethanolic extracts of *P. nigrum* exhibited anti-leishmanial activity, with the hexanic extract being highly selective towards the intracellular stage of the parasite (IC_{50} 14.6 µg/ mL) [56]. Alterations in parasite morphology were also observed, where parasites had an atypical appearance owing to shortening of flagella and cell shrinkage, corroborating the leishmanicidal action of hexanic extract [56]. The compounds also displayed anti-promastigote activity, which was mediated via apoptosis, as evidenced by phosphatidylserine externalization, DNA fragmentation, loss of potential of the mitochondrial membrane and generation of reactive oxygen species. In addition, they reported that *P. nigrum* extracts were devoid of any liver or renal toxicity to the host [72].

The extracts of *P. amalago* L. leaves, obtained by the conventional maceration method with supercritical carbon dioxide (SFE-CO_2) and compressed propane under different conditions, were tested against the promastigote and intracellular amastigote forms of *L. (L.) amazonensis*. SFE-CO2 extracts (313 K; 12.55 MPa) showed the highest anti-leishmanial activity with IC_{50} values of 16 and 7 µg / mL against promastigote and amastigote forms, respectively, and low cytotoxicity to the host cell (CC_{50} value of 93 µg/ml) [31]. The authors indicated that extraction with supercritical fluid using carbon dioxide may be an appropriate method, due to the rapid production of drugs without toxic waste, considering the current requirement for industries to make products without generating organic solvent residues [73].

The methanol extract from *P. betle* was capable of selectively inhibiting promastigotes (IC_{50} 11.2 µg/ mL) and intracellular amastigotes (IC_{50} 9.31 µg/ mL) of *L. donovani*, by accelerating mitochondria-targeted apoptotic events via the generation of reactive oxygen species, without any cytotoxicity towards macrophages [38].

In general, the studies used in this review reported only on the anti-proliferative effects of extracts or fractions. As such, although the results for these compounds are promising, few studies have carried out tests to elucidate the mechanisms of action or the microbicidal response of macrophages to treatment. The limited scope of these studies may be due to restrictions on the use of animals, the high costs involved in further research or the lack of promise of the results obtained for subsequent studies. Therefore, it is not yet clear how these compounds act and detailed assessments of their mechanisms of action are required. Furthermore, few studies have employed bioguided fractionation for the elucidation of the chemical constituents of extracts, which may be important to assist in the identification of substances or potential groups of compounds that, when isolated, can be used against leishmaniasis.

3.5 Isolated Compounds

In this review, 59 chemical compounds, isolated from *Piper* plants, were investigated for their anti-leishmanial activities. The active compounds that have been isolated and identified include simple benzoic acid derivatives (14), amides (14), lignans (7), dihydrochalcones (5), chalcones (3), phenylpropanoids (3), caffeic acids (3), alkaloids (2), flavonoid (1), an aromatic alkene (1), a cyclopentenedione (1), a monoterpene ester (1), an unsaturated fatty acid (1), a neolignan (1), a sesquiterpene (1) and an alkenylphenol (1). These compounds are arranged in (Table 2) to provide information regarding sequences, chemical names, classes, and references.

Compounds isolated from the n-hexane fraction of *Piper longum* fruits showed significant strong activity against axenic amastigotes, compared to promastigotes forms of *L. donovani* (Table 1); however, a new alkaloid amide, piperlongumide (33), was the most potent compound for the two evolutive forms, promastigotes (IC_{50} of 9 µg/mL) and axenic amastigotes (IC_{50} of 2.81 µg/mL) [52]. Studies suggest that amide can induce oxidative stress in leishmania-infected macrophages and

Leishmania parasites are also known to be sensitive to the generation of large amounts of reactive oxygen species, leading to oxidative stress and apoptosis [74].

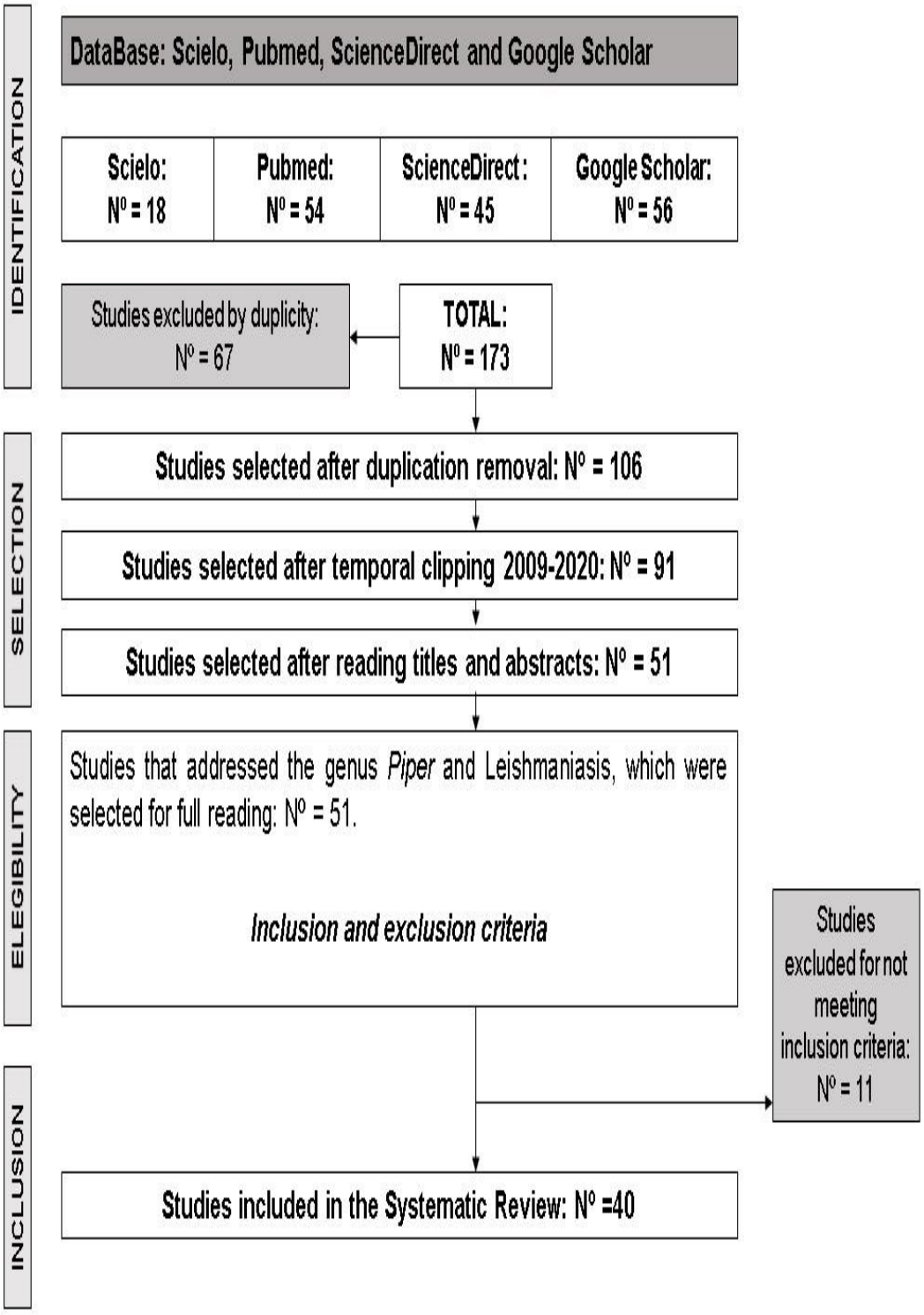

Fig. 1. Flow diagram of the identification and selection of articles for the systematic review of the genus *Piper*

Table 1. Studies on the leishmanicidal activity of the genus *Piper* included in this systematic review

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
					PRO	AMA	
P. aduncum L.	Leaves	Brazil	Dillapiole (1)	*L. amazonensis* *L. braziliensis*	69.3 59.4	-	[25]
P. aduncum L.	Leaves	Brazil	Adunchalcone (2)	*L. amazonensis* *L. braziliensis* *L. shawi* *L. chagasi*	11.03 26.70 11.26 107.31	-	[26]
P. aduncum L.	Leaves	Brazil	Essential oil	*L. amazonensis*	25.9	36.2	[27]
P. aduncum L.	Leaves	Brazil	Essential oil	*L. braziliensis*	77.9	-	[28]
P. aduncum var. ossanum (C. DC.) Saralegui	Leaves	Cuba	Essential oil	*L. infantum* *L. amazonensis*	32.5 19.3	-	[29]
P. aduncum L.	Leaves	Bolivia	3-(3,7-dimethyl-2,6-octadienyl)-4-methoxy-benzoic acid (3) 4-hydroxy-3-(3,7-dimethyl-2,6-octadie-nyl)benzoic acid (4) 4-hydroxy-3-(3- methyl-1-oxo-2-butenyl) - 5-(3-methyl-2-butenyl)benzoic acid (5)	*L. amazonensis* *L. braziliensis* *L. donovani*	(3) 50.1 6.5 50.3 (4) 27.0 27.0 27.0 (5) 17.8 17.8 17.8	-	[30]
P. amalago L.	Leaves	Brazil	SFE-CO_2 313 K and, 12.55 Mpa SFE-CO_2 333 K and, 20.5 Mpa Compressed propane 293 K and, 15.0 Mpa Compressed propane 313 K and, 15.0 Mpa Compressed propane 333 K and, 15.0	*L. amazonensis*	16 27.9 29.8 23.5 34.4	7 13.65 22.85 12.85 23.65	[31]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
			Mpa				
			Chloroform extract		15.0	13.6	
P. amalago L.	Leaves	Brazil	N-[7-(3',4'- methylenedioxyphenyl)-2(Z),4(Z)-heptadienoyl]pyrrolidine(6)	*L. amazonensis*	20	20.5	[32]
			N-[7-(3',4'-methylenedioxyphenyl)-2(E),4(E)-heptadienoyl]pyrrolidine(7)		15	14.5	
P. angustifolium Ruiz & Pav.	Leaves	Brazil	Essential oil	*L. infantum*	-	1.43	[33]
P. auritum Kunth	Aerial parts	Cuba	Essential oil	*L. major*	29.1	-	[34]
				L. mexicana	63.3	-	
				L. braziliensis	52.1	-	
				L. donovani	12.8	22.3	
P. auritum Kunth	Aerial parts	Colombia	Essential oil	*L. infantum*	>100	>100	[35]
P. auritum Kunth	Leaves	Costa Rica	Ethanolic extract	*Leishmania sp.*	60	-	[36]
P. auritum Kunth	Fruits	Costa Rica	Trans-Z-α-bisabolene (8)	*Leishmania sp.*	50	-	[37]
			Safrol (9)		0.0		
P. betle L.	Leaves	India	Methanolic extracts	*L. donovani*	11.2	9.31	[38]
P. bogotense C.DC.	Aerial parts	Colombia	Essential oil	*L. infantum*	>100	>100	[35]
P. brachypodon (Benth.) C. DC.	Aerial parts	Colombia	Essential oil	*L. infantum*	23.43	>100	[35]
P. brachypodon (Benth.) C. DC.	Aerial parts	Colombia	Essential oil	*L. infantum*	23.68	>100	[35]
P. bredemeyeri J.Jacq.	Aerial parts	Colombia	Essential oil	*L. infantum*	>100	>100	[35]
P. carniconnectivum C. DC	Roots	Brazil	2-[1-hydroxy-3-phenyl-(Z,2E)-2-propenylidene]-4- methyl-4-cyclopentene-1,3-dione (10)	*L. amazonensis*	4.4	-	[39]
P. cernuum Vell.	Leaves	Brazil	Essential oil	*L. amazonensis*	27.1	>200	[27]
P. chaba Hunter	Roots	Bangladesh	Petroleum ether extract	*L. donovani*	32	-	[40]
			Chloroform extract		34		
			Bornyl piperate (11)		16		

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
			Piperlonguminine (12)		30		
P. claussenianum (Miq.) C. DC.	Leaves Inflorescences	Brazil	Essential oils	*L. amazonensis*	30.4 1328.0	-	[41]
P. crassinervium Kunth	Leaves	Peru	Ethanolic extract	*L. amazonensis*	-	25.8	[42]
P. cubeba Bojer	Fruits	Brazil	Essential oil	*L. amazonensis*	326.5	-	[43]
P. cumanense Kunth	N.I.	Colombia	Ethanolic extract Aqueous Extract Butanol fraction Dichloromethane fraction Hexan Fraction Methanolic fraction	*L. panamensis*	382.95 >500 >500 >500 244.90 241.90	-	[44]
P. daniel-gonzalezii Trel.	Stems Leaves	Colombia	Degreasing fraction Hexane fraction Dichloromethane fraction Ethyl acetate fraction Ethanol extract of leaves Ethanol extract of stems	*L. panamensis*	-	>100 38.5 >100 >100 55.6 >100	[45]
P. demeraranum (Miq.) C.DC.	Leaves	Brazil	Essential oil	*L. amazonensis* *L. guyanensis*	86.0 22.7	78 -	[46]
P. dennisii Trel.	Leaves	Peru	Ethanolic extract	*L. amazonensis*	-	10	[42]
P. dennisii Trel.	Leaves	Peru	Dennisic acid A (13) Dennisic acid B (14) Piperaduncin C (15) 2′,6′-dihydroxy- 4′-methoxydihydrochalcone (16) 4,2′,6′-trihydroxy-4′-methoxydihydrochalcone (17) 4-hydroxy-3,5-bis(3- methyl-2-butenyl)-benzoic acid (18) 7,8 3,5-bis(3-methyl- 2-butenyl)-4-methoxybenzoic acid (19)	*L. amazonensis*	-	56.9 >89 39.7 183.4 61.4 261.5 244.6 112.1 199.9 20.8	[47]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
			4-hydroxy-3-(3- methyl-2-butenoyl)-5-(3-methyl-2-butenyl)-benzoic acid (20) 2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-chromene-6-carboxylic acid (21) 3-(3′,7′-dimethyl-2′,6′-octadien- yl)-4-methoxybenzoic acid (22)				
P. diospyrifolium Kunth	Leaves	Brazil	Essential oil	*L. amazonensis*	13.5	76.1	[27]
P. divaricatum G. Mey.	Aerial parts	Colombia	Essential oil	*L. infantum*	73.29	>100	[35]
P. duckei C. DC.	Leaves	Brazil	Essential oil	*L. amazonensis* *L. guyanensis*	46.0 15.2	42.4 -	[46]
P. gaudichaudianum (Kunth) Kunth ex Steud.	Leaves	Brazil	Essential oil	*L. amazonensis*	93.5	-	[27]
P. heterophyllum Ruiz & Pav.	Leaves	Bolivia	3-[(2E,6E,10E)-11-carboxy-3,7,15-trimethyl-2,6,10,14- hexadecatetraenyl)-4,5-dihydroxybenzoic acid (23) 3-[(2E,6E,10E)-11-carboxy-13-hydroxy-3,7,15-trimethyl- 2,6,10,14-hexadecatetraenyl]-4,5-dihydroxybenzoic acid (24) 3-[(2E,6E,10E)-11-carboxy-14-hydroxy-3,7,15-trimethyl- 2,6,10,15-hexadecatetraenyl]-4,5-dihydroxybenzoic acid (25) 4,5-dihydroxy-3-(E,E,E-11-formyl-3,7,15-trimethyl-hexadeca-2,6,10,14-tetraenyl)benzoic acid (26) 3,4-dihydroxy-5-(E,E,E-3,7,11,15-tet-ramethyl-hexadeca-2,6,10,14-tetraenyl)benzoic acid (27)	*L. amazonensis* *L. braziliensis* *L.donovani*	(23) >100 >100 >100 (24) 63.1 40.7 46.8 (25) 64.2 39.0 43.9 (26) 77.0 68.5 53.9 (27)	-	[29]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
			4-hydroxy-3-(E,E,E-3,7,11,15-tetramethyl-hexadeca- 2,6,10,14-tetraenyl)benzoic acid (28)		56.1 33.7 42.9 (28) 56.8 44.5 41.8		
P. hispidum Sw.	Leaves	Peru	Ethanolic extract Methanolic fraction N-2-(30,40,50-trimethoxyphenyl)ethyl-2-hydroxybenzamide (29) 2'-hydroxy-3',4',6'-trimethoxychalcone (30) Cardamomin (2',4'-dihydroxy-6'-methoxychalcone) (31) Pinocembrin (5,7-dihydroxyflavanone) (32)	*L. amazonensis*		6.3 5.1 >40 0.8 8 >40	[48]
P. hispidum Sw.	Leaves	French Guiana	Essential oil	*L. amazonensis*		3.4	[49]
P. holtonii C.DC.	N.I.	Colombia	Ethanolic extract Aqueous Extract Butanol fraction Dichloromethane fraction Hexane Fraction Methanolic fraction	*L. panamensis*	280.95 >500 225.90 55.30 418.30 311.50	-	[44]
P. jacquemontianum Kunth	Leaves	Guatemala	Dichloromethane Extract Methane Extract	*L. amazonensis* *L. braziliensis* *L. donovani*	CH_2Cl_2 >1000 102.8 102.8 MeOH 61 20.4 20.8	-	[50]
P. lanceifolium	Aerial	Colombia	Essential oil	*L. infantum*	37.81	>100	[35]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
Kunth	parts						
P. longum L.	Spike	India	Ethanolic extract	*L. donovani*	0.5	-	[51]
P. longum L.	Leaves	India	Piperlongumide (33) 1-(3,4-methylene-dioxyphenyl)-1*E* tetradecene (34) piperlongimin A [2E-N-isobu- tyl-hexadecenamide] (35) 2E,4E-N-isobutyl-octadecenamide (36) piperlongimin B [2E-octadecenoylpiperidine](37) 2E,4E-N-isobu- tyl-dodecenamide (38) 2E,4E,12E,13-(3,4-methylenedioxy-phenyl)-trideca-trienoic acid isobutyl (39)	*L. donovani*	9.12 11.85 14.90 14.15 14.85 14.21 14.85	2.81 3.94 6.15 5.10 4.65 5.60 5.11	[52]
P. malacophyllum (C.Presl) C.DC.	Leaves	Brazil	Gibbilimbol B (40)	*L. infantum*	23.32	22.06	[53]
P. marginatum Jacq.	Aerial parts	Colombia	Essential oil	*L. infantum*	88.7	>100	[35]
P. marginatum Jacq.	Leaves	Brazil	Essential oil Ethanolic extract Methanolic fraction Hexane fraction Ethyl acetate fraction Dichloromethane fraction	*L. amazonensis*	7.9 3.1 0.9 1.0 1.7 7.7	0.6 1.2 15.5 8.2 81.5 17.4	[54]
P. mikanianum (Kunth) Steud.	Leaves	Brazil	Essential oil	*L. amazonensis*	>100	-	[27]
P. mosenii C. DC.	Leaves	Brazil	Essential oil	*L. amazonensis*	17.4	>200	[27]
P. nigrum L.	N.I.	Brazil	Piperine (41)	*L. amazonensis*	14.2	28	[55]
P. nigrum L.	Fruits	India	Hexane extract Ethanolic extract Aqueous extract	*L. donovani*	31.6 37.83 >500	14.63 18.33 >500	[56]
P. nigrum L.	Fruits	Brazil	Piperine (41)	*L. infantum*	3.03	23.98	[57]
P. obrutum Trel. &	Aerial	Colombia	Essential oil	*L. infantum*	35.87	89.02	[35]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
Yunck.	parts						
P. ovatum Vahl	Leaves	Brazil	Hydroalcoholic extract	*L. amazonensis*	60.0	78.5	[58]
			Hexane Fraction		4.7	-	
			Dichloromethane-ethyl acetate Fraction		2.1	24	
			Ethyl acetate Fraction				
			Methanol Fraction		101	-	
			Piperovatine (42)		>1000	-	
			Piperlonguminine (12)		9.5	10	
					2.5	9.0	
P. politaereum Trel.	Leaves	Peru	Ethanolic extract	*L. amazonensis*	-	50.3	[42]
P. pseudoarboreum Yunck.	Leaves	Peru	N-(3,4-Dimethoxy-3-phenyl-propanoyl)-3-chloro-Δ3-pyridin-2-one (43)	*L. amazonensis*	(43)	-	[59]
				L. brasiliensis	3.4		
				L. guyanensis	3.4		
			piplaroxide (44)	*L. infantum*	3.7		
					5.2		
			4,5-dihydropiperlonguminine (45)		(44)		
					71.1		
			(S)-13-hydroxy-octadeca-(9Z,11E,15Z)-trienoic acid (46)		63.6		
					52.1		
					53.8		
					(45)		
					107.3		
					113.5		
					84.4		
					92.4		
					(46)		
					21.1		
					18.7		
					21.8		
					29.6		
P. regnellii (Miq.) C.DC.	Leaves	Brazil	Eupomatenoid-5 (47)	*L. amazonensis*	9	13	[60]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)		References
P. rivinoides Kunth	Leaves	Brazil	Essential oil	*L. amazonensis*	10.9	>200	[27]
P. sanguineispicum Trel.	Leaves	Peru	(S)-1′-Methylbutyl caffeate (48) (S)-1′-Methylhexyl caffeate (49) (S)-1′-Methyloctyl caffeate (50) Sanguinolignan A (51) Sanguinolignan B (52) Sanguinolignan C (53) Sanguinolignan D (54) (7′S)-Parabenzlactone (55) Dihydrocubebin (56) Justiflorinol (57)	*L. amazonensis*	- 	2.0 10.0 1.8 36.7 >130 105.4 69.7 79.4 >140 >140	[61]
P. sanguineispicum Trel.	Leaves Stem	Peru	Ethanolic extract	*L. amazonensis*	-	<10 15	[62]
P. septuplinervium (Miq.) C. DC.	Aerial parts	Colombia	Essential oil	*L. infantum*	30.05	64.8	[35]
P. tuberculatum Jacq.	Fruits	Brazil	3-(3,4,5-trimethoxyphenyl) propanoic acid (58)	*L. amazonensis*	145	-	[63]
P. tuberculatum Jacq.	Fruits	Brazil	Ethanolic extract Hexane fraction Chloroform fraction Ethyl acetate fraction Methanol fraction Pellitorine (59)	*L. guyanensis*	>100 93.89 19.98 >100 >100 26.84	-	[64]
P. umbellatum L.	Leaves	Peru	Ethanolic extract	*L. amazonensis*	-	39.5	[42]
P. variabile C.DC.	Leaves	Guatemala	Dichloromethane Extract Methane Extract	*L. amazonensis* *L. braziliensis* *L. donovani*	CH_2Cl_2 76.3 66.3 55.8 MeOH >1000 >1000 >1000	-	[50]

Scientific name *Piper*	Plant part	Country	Compound/Extract	*Leishmania* species	Results – IC_{50} (µg or µM /mL)	References
P. xylosteoides (Kunth) Steud.	Leaves	Brazil	Essential oil	*L. amazonensis*	>100 -	[27]

IC_{50}: half maximal inhibitory concentration; PRO: Promastigote; AMA: Amastigote; N.I.: Not inform

Table 2. Compounds isolated from the genus *Piper* that have been tested for antileishmanial activity

Code	Chemical substance	Class	References
1	Dillapiole	Phenylpropanoid	[25]
2	Adunchalcone	Chalcone	[26]
3	3-(3,7-dimethyl-2,6-octadienyl)-4-methoxy-benzoic acid	Simple benzoic acid derivatives	[30]
4	4-hydroxy-3-(3,7-dimethyl-2,6-octadie- nyl)benzoic acid	Simple benzoic acid derivatives	[30]
5	4-hydroxy-3-(3- methyl-1-oxo-2-butenyl)-5-(3-methyl-2-butenyl)benzoic acid	Simple benzoic acid derivatives	[30]
6	N-[7-(3',4'- methylenedioxyphenyl)-2(Z),4(Z)-heptadienoyl]pyrrolidine	Amide	[32]
7	N-[7-(3',4'-methylenedioxyphenyl)- 2(E),4(E)-heptadienoyl]pyrrolidine	Amide	[32]
8	Trans-Z-α-bisabolene	Sesquiterpene	[37]
9	Safrol	Phenylpropanoid	[37]
10	2-[1-hydroxy-3-phenyl-(Z,2E)-2-propenylidene] -4-methyl-4-cyclopentene-1,3-dione	Cyclopentenedione	[39]
11	Bornyl piperate	Monoterpene ester	[40]
12	Piperlonguminine	Alkaloid	[40, 58]
13	Dennisic acid A	Dihydrochalcone	[47]
14	Dennisic acid B	Dihydrochalcone	[47]
15	Piperaduncin C	Dihydrochalcone	[47]
16	2',6'-dihydroxy-4'-methoxydihydrochalcone	Dihydrochalcone	[47]
17	4,2',6'-trihydroxy-4'- methoxydihydrochalcone	Dihydrochalcone	[47]
18	4-hydroxy-3,5-bis(3- methyl-2-butenyl)-benzoic acid	Simple benzoic acid derivatives	[47]
19	7,8 3,5-bis(3-methyl- 2-butenyl)-4-methoxybenzoic acid	Simple benzoic acid derivatives	[47]
20	4-hydroxy-3-(3- methyl-2-butenoyl)-5-(3-methyl-2-butenyl)-benzoic acid	Simple benzoic acid derivatives	[47]
21	2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-chromene-6-carboxylic acid	Simple benzoic acid derivatives	[47]
22	3-(3',7'-dimethyl-2',6'-octadien- yl)-4-methoxybenzoic acid	Simple benzoic acid derivatives	[47]
23	3-[(2E,6E,10E)-11-carboxy-3,7,15-trimethyl-2,6,10,14- hexadecatetraenyl)-4,5-dihydroxybenzoic acid	Simple benzoic acid derivatives	[29]
24	3-[(2E,6E,10E)-11-carboxy-13-hydroxy-3,7,15-trimethyl- 2,6,10,14-hexadecatetraenyl]-4,5-dihydroxybenzoic acid	Simple benzoic acid derivatives	[29]

Code	Chemical substance	Class	References
25	3-[(2E,6E,10E)-11-carboxy-14-hydroxy-3,7,15-trimethyl- 2,6,10,15-hexadecatetraenyl]-4,5-dihydroxybenzoic acid	Simple benzoic acid derivatives	[29]
26	4,5-dihydroxy-3-(E,E,E-11-formyl-3,7,15-trimethyl-hexadeca-2,6,10,14-tetraenyl)benzoic acid	Simple benzoic acid derivatives	[29]
27	3,4-dihydroxy-5-(E,E,E-3,7,11,15-tet- ramethyl-hexadeca-2,6,10,14-tetraenyl)benzoic acid	Simple benzoic acid derivatives	[29]
28	4-hydroxy-3-(E,E,E-3,7,11,15-tetramethyl-hexadeca- 2,6,10,14-tetraenyl)benzoic acid	Simple benzoic acid derivatives	[29]
29	N-2-(30,40,50-trimethoxyphenyl)ethyl-2-hydroxybenzamide	Amide	[48]
30	2'-hydroxy-3',4',6'-trimethoxychalcone	Chalcone	[48]
31	Cardamomin (2',4'-dihydroxy-6'-methoxychalcone)	Chalcone	[48]
32	Pinocembrin (5,7-dihydroxyflavanone)	Flavonoid	[48]
33	Piperlongumide	Amide	[52]
34	1-(3,4-methylene-dioxyphenyl)-1E tetradecene	Aromatic alkene	[52]
35	Piperlongimin A[2E-N-isobu- tyl-hexadecenamide]	Amide	[52]
36	2E,4E-N-isobutyl-octadecenamide	Amide	[52]
37	Piperlongimin B [2E-octadecenoylpiperidine]	Amide	[52]
38	2E,4E-N-isobu- tyl-dodecenamide	Amide	[52]
39	2E,4E,12E,13-(3,4-methylenedioxy-phenyl)-trideca-trienoic acid isobutyl	Amide	[52]
40	Gibbilimbol B	Alkenylphenol	[53]
41	Piperine	Amide	[55, 57]
42	Piperovatine	Amide	[58]
43	N-(3,4-Dimethoxy-3-phenyl-propanoyl)-3-chloro-Δ3-pyridin-2-one	Amide	[59]
44	Piplaroxide	Amide	[59]
45	4,5-dihydropiperlonguminine	Amide	[59]
46	(S)-13-hydroxy-octadeca-(9Z,11E,15Z)-trienoic acid	Unsaturated fatty acid	[59]
47	Eupomatenoid-5	Neolignan	[60]
48	(S)-1′-Methylbutyl caffeate	Caffeic Acid derivatives	[61]
49	(S)-1′-Methylhexyl caffeate	Caffeic Acid derivatives	[61]
50	(S)-1′-Methyloctyl caffeate	Caffeic Acid derivatives	[61]
51	Sanguinolignan A	Lignan	[61]
52	Sanguinolignan B	Lignan	[61]
53	Sanguinolignan C	Lignan	[61]
54	Sanguinolignan D	Lignan	[61]
55	(7'S)-Parabenzlactone	Lignan	[61]
56	Dihydrocubebin	Lignan	[61]

Code	Chemical substance	Class	References
57	Justiflorinol	Lignan	[61]
58	3-(3,4,5-trimethoxyphenyl) propanoic acid	Phenylpropanoid derivative	[63]
59	Pellitorine	Alkaloid	[64]

Caffeic acid esters (48-50) isolated from *P. sanguineispicum* showed leishmanicidal activities against axenic amastigotes from *L. (L.) amazonensis* (IC_{50} of 1.8 to 10 µM), with moderate cytotoxicity in murine macrophages [61]. In a study with compounds isolated from bioassay-guided fractionation of the ethanol extract of *P. hispidum* leaves and tested against axenic amastigotes of *L. (L.) amazonensis*, two chalcones demonstrated strong activities with IC_{50} values of 0.8 µM (30) and 8 µM (31), and moderate toxicity against peritoneal macrophages (CC_{50} = 1.6 and 18.2 µM, respectively) [48]. Chalcones are a group of naturally occurring chemical compounds that have different bioactivities, including antimicrobial [75], antifungal [76], anticancer [77], antiviral [78], anti-inflammatory [79] and anti-parasitic [80] activities. Different studies with *Leishmania* reported that the target organelle of chalcones is the parasite mitochondria, where they inhibit its function [81]. The *Leishmania* protozoan has only a single mitochondrion with highly condensed DNA, distributed throughout the body; changes in this organelle induce cellular damage, leading to the process of cell death, constituting a main target for new drugs [82].

3.6 Limitations and Future Perspectives

This systematic review identified that, of the known *Piper* species, only 46 have been investigated for their leishmanicidal activities. To date, the *P. aduncum* species has been most studied since it is used in traditional medicine to treat intestinal disorders and heal wounds [83]. Various biological activities have been reported for this species, including insect repellent activity [84], antihelmintic activity against the nematode *Haemonchus contortus* [85], antifungal and antibacterial activities [86], and inhibition of the expression of proinflammatory cytokines (IL-12p40 and IL-6) in bone marrow-derived dendritic cells [87], thus demonstrating the broad range of biological effects of this plant species. *In vitro* studies of the biological activities of the genus *Piper* are still prevalent, indicating the existence of limitations for carrying out *in vivo* and toxicological tests [14]. Furthermore, researchers evidently continue to search for new alternatives for the treatment of leishmaniasis. However, we note that most tests have been performed with the promastigote form of *Leishmania*, requiring more studies to investigate the anti-amastigote activities of plant-derived compounds, since the amastigote form causes leishmanial-associated morbidity and mortality and is the main target of any leishmaniasis chemotherapy. Since promastigotes are more susceptible to drug-induced effects than amastigotes, the use of promastigotes in assays should be considered as preliminary. Use of an additional intracellular assay should provide more relevant information on compound efficacy [88]. Most studies included in this systematic review, direct counting was commonly used as the methodology of choice to evaluate anti-promastigote and anti-amastigote activities, together with the use of axenic amastigotes. Differences in drug sensitivities exist between axenic amastigotes and intracellular amastigotes [89]. As *Leishmania* is an intracellular pathogen, the use of an intracellular assay to confirm the effects of potential drugs is recommended. The abundance of different experimental designs complicates the interpretation of drug susceptibility and interlaboratory comparisons [66].

4. CONCLUSION

This systematic review finds that plants of the genus *Piper* have potential activity against leishmaniasis. We suggest that the mechanisms of action of these compounds should be further elucidated to enable the development of drugs based on the essential oils, extracts, and chemical constituents of this genus, with a view to discovering new bioactive compounds for the treatment of leishmaniasis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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