Parasitology

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Effect of essential oils on *Leishmania amazonensis*: a systematic review

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Review

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Abstract

This systematic review investigated the evidence for the therapeutic potential of essential oils (EOs) against *Leishmania amazonensis*. We searched available scientific publications from 2005 to 2019 in the PubMed and Web of Science electronic databases, according to PRISMA statement. The search strategy utilized descriptors and free terms. The EOs effect of 35 species of plants identified in this systematic review study, 45.7% had half of the maximal inhibitory concentration (IC_{50}) $10 < IC_{50} \leqslant 50\,\mu g\,mL^{-1}$ and 14.3% had a $10 < IC_{50}\,\mu g\,mL^{-1}$ for promastigote forms of *L. amazonensis*. EOs from *Cymbopogon citratus* species had the lowest IC_{50} ($1.7\,\mu g\,mL^{-1}$). Among the plant species analyzed for activity against intracellular amastigote forms of *L. amazonensis*, 39.4% had an IC_{50} $10 < IC_{50} \leqslant 50\,\mu g\,mL^{-1}$, and 33.3% had an IC_{50} $10 < IC_{50}\,\mu g\,mL^{-1}$. *Aloysia gratissima* EO showed the lowest IC_{50} ($0.16\,\mu g\,mL^{-1}$) for intracellular amastigotes. EOs of *Chenopodium ambrosioides*, *Copaifera martii* and *Carapa guianensis*, administered by the oral route, were effective in reducing parasitic load and lesion volume in *L. amazonensis*-infected BALB/c mice. EOs of *Bixa orellana* and *C. ambrosioides* were effective when administered intraperitoneally. Most of the studies analyzed *in vitro* and *in vivo* for the risk of bias showed moderate methodological quality. These results indicate a stimulus for the development of new phytotherapy drugs for leishmaniasis treatment.

Introduction

Leishmaniasis is a neglected disease of high prevalence and distribution directly linked to social, environmental and climatological factors. It is endemic in 98 countries, with 350 million people at risk, and is the cause of 50 000 deaths every year worldwide (Alvar *et al.*, 2012; World Health Organization, 2019). The majority of cutaneous leishmaniasis cases occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic (World Health Organization, 2019).

The epidemiology of cutaneous leishmaniasis in the Americas is complex, with multiple circulating *Leishmania* species in the same geographical area, several reservoirs, hosts and sand fly vectors, as well as variable clinical manifestations and responses to therapy (Burza *et al.*, 2018). The genetic complexity of the causative agents of leishmaniasis is vast, as shown by studies on the taxonomy of these trypanosomatids through molecular analysis of V7V8 SSU rRNA, Hsp70 and gGAPDH genes (Jirku *et al.*, 2012; Espinosa *et al.*, 2018); this may translate to the variability in clinical manifestations of the disease. American cutaneous leishmaniasis (ACL) is associated with at least 15 *Leishmania* species belonging to the subgenera, *Viannia*, *Leishmania* and *Mundinia*, allocated into the subfamily Leishmaniinae (Espinosa *et al.*, 2018; Silveira, 2019). *Leishmania braziliensis* (subgenera *Viannia*) and *Leishmania amazonensis* (subgenera *Leishmania*) are the dominant pathogenic species in the Americas, and both are involved in cutaneous leishmaniasis which usually presents as a self-limited ulcer that heals in 3–18 months. *Leishmania braziliensis* is also involved in mucosal leishmaniasis, a potentially life-threatening condition that occurs in up to 10% of patients (Burza *et al.*, 2018).

Besides, *L. amazonensis* is responsible for anergic diffuse cutaneous leishmaniasis (DCL), accounting for nearly 1% of all ACL cases each year in Brazil (de Lima *et al.*, 2014; Brasil, Ministério da Saúde, 2017; Machado *et al.*, 2019). The anergic DCL is characterized by massive dermal infiltrates, is chronic with frequent relapses, and presents with clinical, immunological, parasitological, anatomopathological and therapeutic aspects different from other forms of ACL (Costa *et al.*, 2009). *Leishmania amazonensis* infection induces inhibition of the delayed type hypersensitivity of skin, low production of interferon γ (IFN-γ) and high production of interleukin-10 (IL-10) and transforming growth factor-β, leading to inefficient activation of

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infected macrophages and non-response to conventional treatment (Silveira *et al*., 1991, 1998; Silveira, 2019).

The standard treatment comprises of pentavalent antimonials, liposomal amphotericin B, pentamidines and miltefosine (Pelissari *et al*., 2011). These commonly used drugs are toxic, resulting in severe side-effects, such as pancreatitis, leukopenia and more importantly, cardiac arrhythmia (Machado *et al*., 2012). Standard drug therapy is further compounded with high costs and requires administration in a hospital environment, leading patients to discontinue treatment. Parasite resistance to antimonials has been reported, as well as disease recurrence (Sen and Chatterjee, 2011). To achieve control of leishmaniasis, the discovery of new potentially efficient and safer active compounds is necessary.

Diseases have been treated using plants since the dawn of humankind, resulting in the discovery of many bioactive substances, which are the origin for almost half of the existent drugs (Lam, 2007; Ganesan, 2008). Scientific investigations using plants are fundamental, not only for the discovery of new active substances with fewer side-effects, more efficiency and lower cost, but also to amplify knowledge on biodiversity. Much phytochemical research has been conducted employing extracts, essential oils (EOs) and their fractionated compounds. EOs are secondary substances of plants; they have a complex composition, with many compounds, which confer multiple actions to EOs. Studies have demonstrated antileishmanial activity of EOs or isolated compounds from medicinal plants on *Leishmania* species (de Lima *et al*., 2014; Islamuddin *et al*., 2014). Additionally, it is observed that there is selectivity in relation to the species of *Leishmania* on which EOs act. Thus, the EO of *Nectandra hihua* was 121-times more active for *L. infantum* than for *L. amazonensis* (Bosquiroli *et al*., 2017). The EO of *Piper demeraranum* was 3.8 times more active for *L. amazonensis* than for *L. guyanensis* (Moura do Carmo *et al*., 2012).

Considering the severity of the disease induced by *L. amazonensis* and the potential of EOs as a source of new bioactive drugs, we systematically investigated previous studies on *in vivo* and *in vitro* activity of EOs on this species.

Methods

Search strategy

In-depth search was conducted on PubMed and Web of Science databases to retrieve articles describing the area of interest, published between April 2005 and April 2019. PRISMA statement recommendations were followed in this systematic review (Moher *et al*., 2015). The descriptors or MeSH terms (Medical Subject Headings) were defined independently by five reviewers of group 1 (CELS, JO, FBPF, MPPS, TVAL, RCLS) and Three specialists (MVCL, MSTM and JJVT). The MeSH terms were divided into three blocks – block 1 ('plant oils', 'volatile oils'); block 2 ('complementary therapies', 'anti-infective agents', 'antiprotozoal agents', 'phytotherapy', 'plants medicinal', 'biological products'); and block 3 ('*Leishmania*', 'leishmaniasis'), and combined to retrieve all potential published articles. The descriptors were also researched in the titles of the articles, in individual or combined pairs to increase research sensitivity.

Articles selection

Inclusion and exclusion criteria: All studies that evaluated the therapeutic effects of EOs against *L. amazonensis* were included. Database filters were used and abstracts were read to select studies. Only original studies published in Portuguese, English, Spanish or French languages with accessible abstracts were included. Through database filtering and abstract readings, experimental studies were selected for the systematic review. We excluded review studies, case reports, editorials, comparisons, editor comments, clinical assays, letters, news and guidelines. Articles not found through databases and those that did not meet the selection criteria were not selected.

Quality evaluation: At this stage, full-text selected articles, which constituted the articles of potential interest, were retrieved and randomly distributed to researchers in group 1. The final validation of the selected publications was conducted by consensus among three judges in group 2 (MVCL, MSTM and JJVT). The references listed in each paper were also explored for potential articles of interest not identified in the initial phase.

Data extraction

For the organization and structuring of the tables, researchers in group 1 extracted the content of interest from the papers with the support of group 2. This strategy improved the quality and precision of content extracted from each article. To reduce the risk of bias regarding the content extracted from the papers, a standardized instrument was used for the researchers. The following relevant information was extracted and inserted in the table: family, plants species, *in vitro* results (promastigotes, amastigotes), *in vivo* results and references. Researchers critically validated the information from each selected paper in pairs.

Risk of bias

The quality of the selected publications was carried out through the risk of bias in individual studies independently by two researcher specialists (JJVT and MVCL). For analysis of 23 studies exclusively *in vitro*, we use a checklist consisting of 15 domains, based on the CONSORT (Consolidated Standards of Reporting Trials) guidelines (Faggion, 2012). For analysis of two studies exclusively *in vivo*, we use the Rob (Risk of Bias) tool for animal intervention studies (Systematic Review Center for Laboratory Animal Experimentation – SYRCLE RoB tool), consisting of 12 domains (Hooijmans *et al*., 2014), characterized as an adjusted risk model Cochrane of the bias tool. Six studies presented experiments both *in vivo* and *in vitro*. Currently, no checklist allows evaluating *in vitro* and *in vivo* studies simultaneously. Thus, we apply the checklists separately for each design model. The analysis of the methodological quality of each publication was carried out according to the scores from zero to ten for *in vivo* studies and from zero to 15 for *in vivo* studies, where high scores allowed the high quality of publications.

Results

Published studies were identified in two electronic databases. During the analysis of titles and abstracts of 436 publications by the reviewers, 146 duplicated articles were excluded. Following the application of exclusion criteria, 243 studies were excluded, and 47 were selected since they corresponded to the inclusion criteria, and 14 were excluded for not meeting goals of the study (Fig. 1). The 33 confirmed primary studies were printed in PDF format, analysed thoroughly for data extraction, and were organized and structured (Table 1). This study identified 46 plants species, and all were pre-clinical and experimental. The majority (75.7%) being *in vitro* studies, 6.1% *in vivo* and 18.2% *in vitro* and *in vivo*. Several of the *in vitro* studies simultaneously investigated the effect of EO on promastigote (35 plant species) and amastigote (33 plant species) forms of *L. amazonensis*.

Among the EOs of 35 plant species identified in this systematic review study, 45.7% had a $10 < IC_{50} \leqslant 50\,\mu g\,mL^{-1}$ and 14.3% had

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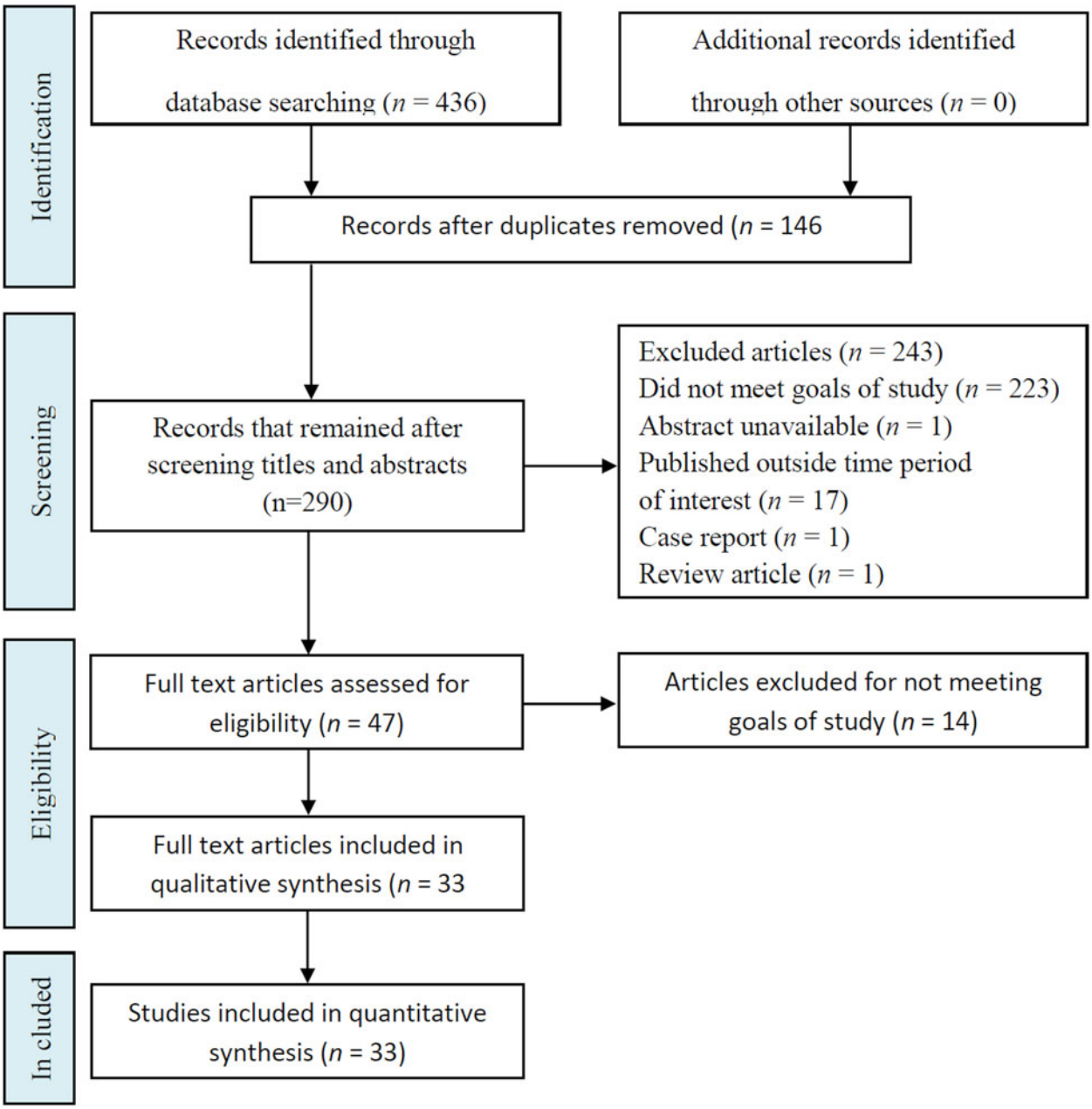


Fig. 1. Flowchart of the different phases of articles included in systematic review.

a $10 < IC_{50}\,\mu g\,mL^{-1}$ for promastigote forms of *L. amazonensis* (Supplementary File S1 and S1a). *Cymbopogon citratus* species had the lowest IC_{50} ($1.7\,\mu g\,mL^{-1}$). However, we identified bioactive agents from ten plant species with IC_{50} values higher than $100\,\mu g\,mL^{-1}$ for *Leishmania* promastigotes. However, 33 species of plants investigated for activity against intracellular amastigote forms of *L. amazonensis*, 39.4% had a $10 < IC_{50} \leqslant 50\,\mu g\,mL^{-1}$ and 33.3% had a $10 < IC_{50}\,\mu g\,mL^{-1}$. The EO of *Aloysia gratissima* showed the lowest IC_{50} ($0.16\,\mu g\,mL^{-1}$) for intracellular amastigotes (Garcia *et al.*, 2018).

The study of *Leishmania* infection in animal models has been described in eight articles, including the study of antileishmanial activity of EOs of six plant species, *Pluchea carolinensis* (Garcia *et al.*, 2017), *Bixa orellana* (Monzote *et al.*, 2014*a*), *C. martii* (dos Santos *et al.*, 2011; Dhorm Pimentel de Moraes *et al.*, 2018), *Tetradenia riparia* (Cardoso *et al.*, 2015), *C. guianensis* (Dhorm Pimentel de Moraes *et al.*, 2018) and *C. ambrosioides* (Monzote *et al.*, 2006, 2007, 2009). Of the 33 publications selected, eight used the *in vivo* model, and the same study used between one and three routes of administration. Among the EOs of these species, EOs of *C. ambrosioides* (Monzote *et al.*, 2009), *C. martii* (dos Santos *et al.*, 2011; Dhorm Pimentel de Moraes *et al.*, 2018) and *C. guianensis* (Dhorm Pimentel de Moraes *et al.*, 2018), when administered by the oral route, were effective in reducing parasitic load and lesion volume in *L. amazonensis*-infected BALB/c mice. EOs of *B. orellana* (Monzote *et al.*, 2014*a*) and *C. ambrosioides* (Monzote *et al.*, 2006) were effective when administered intraperitoneally. EO of *T. riparia*, when given topically, did not alter the volume of lesions, but reduced the parasitic load on the spleen and lymph nodes of *L. amazonensis*-infected BALB/c mice.

Risk of bias assessment

The quality assessments of 33 selected publications were mentioned in Supplementary Tables S2–S5. The score of *in vitro* studies ranged from 5 to 9 out of a total of 15 points (Supplementary File S2 and S3). All *in vitro* studies performed the domains for

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Table 1. Main characteristics of primary studies *in vitro* and *in vivo* with essential oils on *Leishmania amazonensis*

Family	Species plants	Method of essential oils extraction	*In vitro* results		*In vivo* results	Author/year
			Promastigotes IC_{50} ($\mu g\,mL^{-1}$)	Amastigotes IC_{50} ($\mu g\,mL^{-1}$)		
Anacardiaceae	*Myracrodruon urundeuva*	The plant material was air-dried for 7 days. The hydrodistillation used a Clevenger-type apparatus (300 g, 6 h). The EO was dried over anhydrous sodium sulphate, filtered, and stored (dark flask/at 4°C)	EO: 205	EO: 44.5[1]; 104.5[2]	NR	Carvalho *et al.* (2017)
Asteraceae	*Pluchea carolinensis*	The fresh plant sample was collected early in the morning and manually crushed. The crude EO was extracted directly by hydrodistillation for 5 h using a Clevenger-type apparatus	EO: 24.7	EO: 6.2[1]	EO (30 mg kg^{-1}) intralesional: smaller lesion size and parasite burden	Garcia *et al.* (2017)
	Achillea millefolium	The EO oil was obtained by steam distillation of the fresh leaves and flowers, employing Clevenger's apparatus	EO: 7.8 (72 h)	EO: 6.5 [1]	NR	Santos *et al.* (2010)
	Matricaria chamomilla	The EO was extracted by hydrodistillation using a modified Clevenger apparatus. The fresh, chopped leaves (300 g, 2 h) were extracted in three repetitions. The EO was separated by centrifugation at 1100 g/5 min, and was stored in a glass bottle at 4°C, and protected from light	EO: 60.16 (24 h)	NR	NR	Andrade *et al.* (2016)
	Matricaria recutita	The EO was extracted by hydrodistillation (3 h) using Clevenger type apparatus. Flowers of *M. recutita* rinsed in running tap water then air-dried for 14 days were immersed in water and heated to boiling. EO was evaporated and collected in a condenser. The obtained EO was dried over anhydrous sodium sulfate, and stored at −4°C	EO: 10.8 (72 h) (−)-α-bisabolol: 16.0	(−)-α-bisabolol: 5.9[1]	NR	Hajaji *et al.* (2018)
	Mikania micrantha	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 6.8[2]	NR	Houel *et al.* (2015)
	Vernonia brasiliana	The crushed fresh material (roots, leaves and flowers) was submitted to hydrodistillation for 4 h in a Clevenger-type apparatus (50 g of sample/500.0 mL water) The fraction obtained was extracted with dichloromethane. The organic layer was dried (anhydrous magnesium sulphate), and filtered	Leaves: 213; Flower: 112; Roots: 109	NR	NR	Martins *et al.* (2015)
Bixaceae	*Bixa orellana*	The EO was obtained by hydrodistillation of the seeds dried under ventilation by using Clevenger-type equipment	NR	EO: 8.5[1]	EO (30 mg kg^{-1}) for 14 days (intraperitoneal): reduction of parasite load and in the lesion size	Monzote *et al.* (2014*a*)
Burseraceae	*Protium heptaphyllum*	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 34.9[1]; 3.7[2]	NR	Houel *et al.* (2015)

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Table 1. (*Continued.*)

Family	Species plants	Method of essential oils extraction	*In vitro* results		*In vivo* results	Author/year
			Promastigotes IC_{50} ($\mu g\,mL^{-1}$)	Amastigotes IC_{50} ($\mu g\,mL^{-1}$)		
Chenopodiaceae	*Chenopodium ambrosioides*	The EO of the aerial parts of the plant was obtained by distillation, under laboratory conditions, using a Clevenger apparatus	EO: 3.7	EO: 4.6[1]	EO (30 mg kg^{-1}) for 15 days (intraperitoneal route): reduction in the size of the lesion, and suppression of the number of parasites in the infected footpads	Monzote *et al.* (2006)
	C. ambrosioides	The EO of the aerial parts of the plant was obtained by distillation using a Clevenger apparatus	NR	NR	EO (30 mg kg^{-1} day^{-1} intraperitoneal) prevented lesion development and decrease the parasite burden; EO (30 mg kg^{-1} day^{-1} oral route retarded the infection; EO (3% day^{-1} intralesional route) did not show activity	Monzote *et al.* (2007)
	C. ambrosioides	The EO was obtained by distillation of the aerial parts of the plant using a Clevenger's apparatus	NR	NR	EO for 15 days (oral, 150 mg kg^{-1}) was the most effective and better activity	Monzote *et al.* (2009)
	C. ambrosioides	The EO was obtained by distillation of the aerial parts of the plant using a Clevenger's apparatus	EO: 3.7; Ascaridole: 0.1; Carvacrol: 15.3; Caryophyllene: 4.9; EO + Ascaridole: 0.1	EO: 4.6[1]; Ascaridole 0.3[1]; Carvacrol 13.6[1]; Caryophyllene 4.4[1]	NR	Monzote *et al.* (2014*a*, 2014*b*)
Fabaceae	*Copaifera martii*	The *Copaifera* sp. EO was obtained from a producer cooperative (Sena Madureira, Acre, Brazil), and tested for purity and physical and chemical characteristics	Nanocopa: 18 (24 h); 30 (48 h)	IC_{50} inhibition: 30 $\mu g\,mL^{-1}$ 50%; 90 $\mu g\,mL^{-1}$ 90%	Mice treated for 8 weeks: smaller lesions than those on untreated mice	Dhorm Pimentel de Moraes *et al.* (2018)
	C. martii	The copaiba EO was collected from the trunk of *C. martii* tree	EO: 14	NR	Oral route (100 mg kg^{-1} day^{-1}) + topical route (4%, 1 mg mm^{-2}): reduction in the size of the lesions. Topical + subcutaneous routes (100 mg kg^{-1} day^{-1}): not reduction in the size of the lesion	dos Santos *et al.* (2011)
	Vouacapoua americana	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 7.2[2]	NR	Houel *et al.* (2015)
	Pterodon pubescens	Turbo extraction: whole fruits (30 g) were subjected to three consecutive extractions in 99.9% ethanol, and concentrated in a rotary vacuum evaporator. Maceration dynamics: cut fruit (30 g) were submitted to constant mechanical stirring for 3 consecutive days in 99.8% ethanol, and then it was concentrated on a rotary evaporator. The hexane fractions were collected (turboextraction-FHe1 and maceration-FHe2).	FHe1 (FHe, hexane fraction): 41; FHe2: 52.1; EOe3 (EOe, supercritical fluid extract): 26.3; EOe4: 26.3; EOe5: 25.9; EOe6: 28.4; EOe7: 26.4	FHe1: 40.7[1]; EOe3: 33.8[1]; *N.FHe 1* (nanoemulsion, hexane extract): 2.7[1]; *N.EOe3* (nanoemulsion, supercritical extract): 1.9[1]	NR	da Silva Santos *et al.* (2016)

		Supercritical CO_2 extraction ($scCO_2$): the dried fruits were crushed with a knife mill (30 g)				
Lamiaceae	*Tetradenia riparia*	The EO was obtained of fresh leaves (60 g) by steam distillation in a Clevenger apparatus for 3 h with 600 mL of distilled water. The EO was dried with anhydrous sodium sulphate, and stored at 4°C	EO of spring: 15.47; EO of summer: 15.67; EO of autumn: 15.66; EO of winter: 13.31	IC_{50} inhibition by EO at 30 ng mL^{-1}; spring: 43.53%; summer: 32.03%; autumn: 40.54%; winter: 52.49%	Topical route (0.5% or 1%) daily for 8 weeks: reduction of the parasite load in the spleen	Cardoso *et al.* (2015)
	T. riparia	The EO of *T. riparia* fresh leaves was obtained by hydrodistillation for 3 h using a Clevenger-type apparatus. The distilled EO was dried over anhydrous sodium sulphate and stored at −18°C	EO: 2.45; TrROY: 0.03	IC_{50} inhibition by EO at 30 μg mL^{-1}: 65%; IC_{50} inhibition by TrROY at 10 μg mL^{-1}: 48%	NR	Demarchi *et al.* (2015)
	Ocimum canum	The EO was extracted by hydrodistillation using a Clevenger system. 100 g of dry leaves diluted in water (1:10) were boiled to 100°C for 3 h. The EO was dried with anhydrous sodium sulphate	EO: 17.4	EO: 13.1[1]	NR	da Silva *et al.* (2018)
	Origanum vulgare	The aerial parts were dried for 48 h and sprayed in an electric knife mill. The EO was extracted by hydrodistillation using a Clevenger apparatus. The plant diluted in water (1:10) were boiled to 100°C for 3 h. The EO was dried with anhydrous sodium sulphate	EO: 405.5	NR	NR	Teles *et al.* (2019)
	Ocimum gratissimum	Fresh leaves from the plant were cut into pieces and subjected to steam distillation. Then, it was extracted with petroleum ether, which was removed carefully, and the EO was obtained	EO: 135; Eugenol: 80	EO: 100[2]	NR	Ueda-Nakamura *et al.* (2006)
Lauraceae	*Nectandraamazonum*	Fresh material (leaves) from *N. amazonum* was milled and EO was extracted by hydrodistillation in a Clevenger apparatus for 5 h, and dried with anhydrous sodium sulphate	NR	EO: 22.1[1]	NR	Bosquiroli *et al.* (2017)
	N. gardneri	Fresh material (stem bark) from *N. gardneri* was milled and EO was extracted by hydrodistillation in a Clevenger apparatus for 5 h, and dried with anhydrous sodium sulphate	NR	EO: 2.1[1]	NR	
	N. hihua	Fresh material (stem bark) from *N. hihua* was milled and EO was extracted by hydrodistillation in a Clevenger apparatus for 5 h, and dried with anhydrous sodium sulphate	NR	EO: 24.2[1]	NR	
	N. megapotamica	Fresh material (stem bark) from *N. megapotamica* was milled and EO (NMEO1) was extracted by hydrodistillation in a Clevenger apparatus for 5 h, and dried with anhydrous sodium sulphate	NR	EO: 19.0[1]	NR	
	N. megapotamica	Fresh material (leaves) from *N. megapotamica* was milled and EO (NMEO2) was extracted by hydrodistillation in a Clevenger apparatus for 5 h, and dried with anhydrous sodium sulphate	NR	EO: 21.3[1]	NR	
	Cinnamomum zeylanicum	The leaves were dried for 48 h and sprayed in an electric knife mill. The EO was extracted by hydrodistillation using a Clevenger apparatus. The plant diluted in water (1:10) were boiled to 100°C for	EO: >500	NR	NR	Teles *et al.* (2019)

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			Promastigotes IC_{50} ($\mu g\,mL^{-1}$)	Amastigotes IC_{50} ($\mu g\,mL^{-1}$)		
		3 h. The EO was dried with anhydrous sodium sulphate				
	Cryptocarya aschersoniana	Fresh leaves of *C. aschersoniana* (300 g) were subjected to hydrodistillation for 2 h in a Clevenger-type apparatus and collected manually. The extraction procedure was done in triplicate	EO: 4.46	NR	NR	Andrade *et al.* (2018)
Meliaceae	*Carapa guianensis*	The *C. guianensis* (andiroba) EO was obtained from a producer cooperative (Cruzeiro do Sul Acre, Brazil), and tested for purity and physical and chemical characteristics	EO: 590 (24 h); EO: 260 (48 h)	IC_{50} inhibition by EO at 300 $\mu g\,mL^{-1}$: 96%	EO for 8 weeks (100-160 mg kg^{-1}, oral route): amastigotes were less abundant in lesions of treated-mice	Dhorm Pimentel de Moraes *et al.* (2018)
	C. guianensis and 5 fractions	The process of obtaining *C. guianensis* seed oil was an artisanal manner. The fruit was cooked, the andiroba nuts removed, and macerated to obtain the oil	*EO:* did not exhibit leishmanicidal activity; Limonoid-rich fractions: LF3: 10.53; LF4: 25.30; LF5: 56.90	Limonoid-rich fractions: LF3: 27[1]; LF4: 78.42[1]; LF5: 352.2[1]	NR	Oliveira *et al.* (2018)
Myrtaceae	*Syzygium cumini*	Large leaves of *S. cumini* were collected from a mature tree in the flowering stage. Plant material was air-dried for 7 days, cut into small pieces and the EO extracted by hydrodistillation using a Clevenger-type apparatus (300 g, 3 h). The EO was dried (anh. Na_2SO_4), and centrifuged	EO: 60	NR	NR	Dias *et al.* (2013)
	S. cumini	The *S. cumini* EO was extracted of leaves by conventional steam hydrodistillation	EO: 60 α-Pinene: 19.7	EO: 38.1[1]; EO: 43.9[2]; α-Pinene: 15.6[1]; 16.1[2]	NR	Rodrigues *et al.* (2015)
Piperaceae	*Piper rivinoides*	The EOs of *Piper* sp. were obtained from fresh leaves (600 g) by hydrodistillation in a Clevenger apparatus for 4 h with 600 mL of water. The EOs were collected, centrifuged at 5000 rpm/2 min, and stored at −4°C	EO: 10.9	EO: >200[2]	NR	Bernuci *et al.* (2016)
	P. mosenii		EO: 17.4	EO: >200[2]	NR	
	P. cernuum		EO: 27.1	EO: >200[2]	NR	
	P. diospyrifolium		EO: 13.5	EO: 76.1[2]	NR	
	P. arboretum		EO: 15.2	EO: >200[2]	NR	
	P. aduncum		EO: 25.9	EO: 32.2[2]	NR	
	P. gaudichaudianum		EO: 93.5	NR	NR	
	P. xylosteoides		EO: >100	NR	NR	
	P. mikanianum		EO: >100	NR	NR	

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	P. cubeba	The *P. cubeba* EO was obtained by steam distillation was purchased from Suraj Bala Exports	EO: 326.5	NR	NR	Esperandim *et al.* (2013)
	P. claussenianum	The EOs were separately extracted from leaves and inflorescences of fresh and air-dried material. Each fresh and dried sample (about 100 g) was extracted by hydrodistillation for 2 h in a Clevenger-type apparatus. The EOs were dried over anhydrous sodium sulphate and stored at 4°C	EO leaves: 30.4; EO inflorescens: 1.328	NR	NR	Marques *et al.* (2010)
	P. demeraranum	The leaves of *P. demeraranum* (600 g) were dried at room temperature, ground, and submitted to hydrodistillation (4 h) using a modified Clevenger-type apparatus. The EO was collected, and stored in glass flasks at −4°C.	EO: 86	EO: 78[1]	NR	Moura do Carmo *et al.* (2012)
	P. duckei	The EO of *P. duckei* leaves were dried at room temperature, ground, and submitted to hydrodistillation (4 h) using a modified Clevenger-type apparatus. After the end of each distillation, the EO was stored in glass flasks at −4°C	EO: 46	EO: 42[1]	NR	Moura do Carmo *et al.* (2012)
	P. hispidum	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 4.7[1]; EO: 3.4[2]	NR	Houel *et al.* (2015)
Plantaginaceae	*Otacanthus azureus*	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C.	NR	EO: 16.1[1]; EO: 0.7[2]	NR	Houel *et al.* (2015)
Poaceae	*Cymbopogon citratus*	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 5.3[2]	NR	Houel *et al.* (2015)
	C. citratus	The EO was obtained by steam distillation of fresh leaves from the plant, employing Clevenger's apparatus, and was stored at −20°C	EO: 1.7 Citral: 8.0	EO: 3.2[1]; Citral: 25.0[1]	NR	Santin *et al.* (2009)
Rubiaceae	*Mitracarpus frigidus*	The EO was obtained from dried aerial parts of *M. frigidus* by hydrodistillation (3 h) using a Clevenger type apparatus. The EO was stored at 4°C	EO: 89.7	NR	NR	Fabri *et al.*, 2012
Scrophulariaceae	*Achetaria guianensis*	The EO was obtained from fresh parts of the plant by hydrodistillation and stored at −18°C	NR	EO: 6.3[2]	NR	Houel *et al.* (2015)
Verbenaceae	*Lippia sidoides*	The fresh aerial parts of *L. sidoides* (100 g) were submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. The EO were dried over anhydrous sodium sulphate and stored at −20°C	EO: 44.38; Thymol: 19.47	EO: 34.4[1]	NR	de Medeiros *et al.* (2011)
	Aloysia gratissima	Fresh aerial parts (leaves and branches, 1020 g) of *A. gratissima* were hydrodistilled (2 L water) in a modified Clevenger apparatus (6 h). The volatile EO was stored in sealed amber ampoules at −20°C	EO: 25 (48 h); 14 (72 h)	EO: 0.16[1]; Guaiacol: 0.01[1]	NR	Garcia *et al.* (2018)
Zingiberaceae	*Curcuma longa*	The leaves were dried for 48 h and sprayed in an electric knife mill. The EO was extracted by hydrodistillation using a Clevenger apparatus. The plant diluted in water (1:10) were boiled to 100°C for 3 h. The EO was dried with anhydrous sodium sulphate	EO: 308.4	EO: 63.3[1]	NR	Teles *et al.* (2019)

EO, essential oil; IC_{50}, concentration of the EO that reduced the survival of *Leishmania* parasites by 50% compared with untreated parasites; CC_{50}, cytotoxicity to cells 50%; $^{1}IC_{50}$, obtained with intracellular amastigote on peritoneal macrophages of Balb/c mouse infected with *Leishmania*; $^{2}IC_{50}$, obtained with axenic *Leishmania* amastigote; NO, nitric oxide; NR, not reported.

structured abstract, scientific background, and rationale, objectives and/or hypotheses, and intervention of each group. No studies reported allocation sequence generation, allocation concealment mechanism, implementation, outcomes and estimation. Only one publication (Monzote *et al.*, 2014*a*, 2014*b*) applied the blinding domain. The articles with the *in vivo* experimental model had scores ranging from 3 to 7, among ten domains (Supplementary File S4 and S5). All publications developed the domains baseline characteristics, selective outcome reporting, other sources of bias. The allocation sequence generation domain was applied in most publications. The domain blinding of personnel and participants, and random outcome assessment was performed in only one study (dos Santos *et al.*, 2011).

Discussion

Natural plant products are essential sources for drug innovation, and the selection and validation of extracts or molecules with relevant pharmacological action requires strategies for choosing effective bioproducts (Clardy and Walsh, 2004; Cos *et al.*, 2006). In this systematic review, we retrieved studies on EOs of plants that specifically act against *L. amazonensis*, a principal causative agent in cutaneous leishmaniasis. Various screening approaches are available to identify the primary pharmacological actions of natural products on *Leishmania*. Promastigote forms of *Leishmania* multiply in defined culture media, and thus, the determination of IC_{50} of a bioproduct is widely employed among studies. Another interesting bioassay method evaluates the concentration capable of killing 50% of amastigote forms (IC_{50}) in peritoneal macrophages of BALB/c mice infected with *Leishmania* (Cos *et al.*, 2006). Several articles also address the mechanisms of action of these bioproducts by ultrastructural analysis using transmission electron microscope (TEM), investigation of cell death induction and studies on microbicidal metabolites of macrophages, such as nitric oxide (NO) and various cytokines that modulate the immune response. Measurement of the 50% toxicity concentration of bioproducts on murine cells or cell lines (CC_{50}), and the action on erythrocytes allows an assessment of toxicity the bioproducts may be. These studies usually precede animal model trials in which mice are infected, develop the disease and are subsequently treated using various protocols. Each test should contain at least one reference drug to ascertain test performance and proper interpretation of the screening results. Relevant and selective activity is related to IC_{50}-values below 100 $\mu g\,mL^{-1}$ for extracts and below 25 μM for pure compounds (Cos *et al.*, 2006). In this review, we studied the EOs of plant species from the following families:

Anacardiaceae family

Myracrodruon urundeuva: *M. urundeuva* is native to South America and is used in traditional medical practices in Brazil for the treatment of mycoses, candidiasis, bacterial infections and as an anti-inflammatory agent (Pereira *et al.*, 2014). The EO of this species (MuEO) contains monoterpene and sesquiterpene hydrocarbons, the main constituent being β-myrcene (42.46%), followed by α-myrcene (37.23%) and caryophyllene (4.28%). β-myrcene showed anti-*L. amazonensis* activity, as previously reported (Carvalho *et al.*, 2017; Machado *et al.*, 2012), preferably against the intracellular amastigote forms, which are involved in the development of the clinical manifestations of leishmaniasis. In optical microscopy, it was observed that MuEO-induced morphological changes in promastigotes, showing cells with round or spherical shapes, as well as the presence of cell debris (typical of cell lysis), suggesting leishmanicidal activity (Carvalho *et al.*, 2017). MuEO decreased macrophage viability only at high concentrations (>200 $\mu g\,mL^{-1}$). However, cytotoxicity against erythrocytes was low, with no alteration on lysosomal activity or NO production in macrophages, which indicates probable lack of direct involvement in immunomodulatory mechanisms (Carvalho *et al.*, 2017).

Asteraceae family

Achillea millefolium: *A. millefolium*, which is native to Europe, North America and South Australia, is a herbaceous plant with a perennial life cycle and is popularly referred to as 'thousand leaves'. EO from the leaves and flowers of *A. millefolium* was active against *L. amazonensis* with a promising IC_{50} of 6.5 $\mu g\,mL^{-1}$ and CC_{50} of 72.0 $\mu g\,mL^{-1}$ (Santos *et al.*, 2010). In scanning electron microscopy (SEM), the parasites revealed alterations of shape and size, and in TEM, ultrastructural changes in the flagellar membrane, abnormal membrane structures, rupture of the plasma membrane, atypical vacuoles, myelin-like figures and vesicles that resembled autophagic vacuoles were observed (Santos *et al.*, 2010). *Achillea millefolium* extracts have also been reported to possess anti-inflammatory activity (Tadic *et al.*, 2017), which may be related to its content of phenolic compounds, more specifically, dicaffeoylquinic acids, luteolin, apigenin and its glycosides. Additionally, Villalva *et al.* (2019) reported the inhibitory effect of *A. millefolium* fractions on IL-1β, IL-6 and tumour necrosis factor-α (TNF-α) secretion.

Matricaria chamomilla: The gas chromatography-mass spectrometry (GC-MS) analyses for *M. chamomilla* EO identified the main constituents as β-farnesene (52.73%), bisabolol oxide (12.09%), α-farnesene (10.34%) and α-bisabolo (9.83%) (Andrade *et al.*, 2016). This same study compared the IC_{50}/24 h (60.16 $\mu g\,mL^{-1}$) for promastigote forms of *L. amazonensis*, and cytotoxicity against L6 cells (CC_{50}/24 h 173.04 $\mu g\,mL^{-1}$) using the selectivity index (173.04 $\mu g\,mL^{-1}$), and it was considered moderately active ($50 < IC50 \leqslant 150\,\mu g\,mL^{-1}$) (Andrade *et al.*, 2016).

Matricaria recutita: *M. recutita* is native to northern Europe and grows wild in Central European countries, Eastern Europe, western Asia, the Mediterranean region of North Africa and the Americas. Chamomile is one of the plants most cited for medicinal purposes in qualitative studies, for adult or paediatric use (Brasil, Ministério da Saúde, 2015). It has uses mentioned in pharmacopoeias, ethnobotanical studies, folk medicine, complementary and alternative medicine. A bio-guided study conducted by Hajaji *et al.* (2018) identified the mechanism involved in Tunisian chamomile EO leishmanicidal action. The IC_{50} values for *L. amazonensis* promastigote were low and ranging from 10.8 $\mu g\,mL^{-1}$ for the EO to 16.0 $\mu g\,mL^{-1}$ for (−)-α-bisabolol. The CC_{50} on macrophages J774.A1 was 31.9 $\mu g\,mL^{-1}$, and the selectivity index was 5.5. The (−)-α-bisabolol was able to activate a programmed cell death process. This compound induced phosphatidylserine externalization and membrane damage, decrease the mitochondrial membrane potential and total ATP levels in the promastigote of *L. amazonensis* (Hajaji *et al.*, 2018). *Matricaria recutita* and its active compound (−)-α-bisabolol can be a natural potential alternative to the available drugs.

Mikania micrantha: *M. micrantha* is native to Central and South America and is considered as an invasive weed, growing in cattle fields. Laurella *et al.* (2017) isolated and identified four sesquiterpene lactones of the germacranolide type from *M. micrantha* organic extracts: mikanolide, dihydromikanolide and deoxymikanolide scandenolide. They observed that mikanolide and deoxymikanolide showed significant activity against *L. braziliensis* promastigotes, while dihydromikanolide displayed moderate activity. Houel *et al.* (2015) used a strategy to discover bioactive natural products based on bioinspiration, which allows

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the transposition of these desirable properties to a corresponding research field. These authors evaluated the antileishmanial properties of selected anti-dermatophytic plants, looking for a match with antileishmanial activity. *Mikania micrantha* EO showed weak to non-existent activity against selected dermatophytic filamentous fungi but achieved action against *L. amazonensis* axenic amastigotes, while presenting low cytotoxicity to VERO cells and BALB/c mice peritoneal macrophages (Houel *et al.*, 2015).

Pluchea carolinensis: This plant has a broad native distribution, from Mexico and Central America to South America. Garcia *et al.* (2017) described that the EO from aerial parts of *P. carolinensis* contained at least 44 compounds, the main component being selin-11-en-4α-ol. This EO inhibited the growth of promastigote and amastigote forms of *L. amazonensis*, while its cytotoxicity was 5-fold higher for peritoneal macrophages than that for the parasites. In an experimental model of infection, BALB/c mice treated with five doses of the EO (30 mg kg^{-1}) by intralesional route presented reduced lesion size and parasite burden. This potential EO may target specific molecules or pathways in the amastigote form or induce defence mechanisms in the macrophages that contributed to the antileishmanial activity.

Vernonia brasiliana: *V. brasiliana* is a bush tree observed in the Brazilian savannah, also known as 'assa-peixe'. The EO from flowers, roots and leaves of *V. brasiliana* did not display cytotoxicity against Vero (ATCC CCL 81) and RAW 264.7 cell lines, but they showed inhibitory activity against *T. cruzi* (Martins *et al.*, 2015). The only study about *V. brasiliana*, by Martins *et al.* (2015) showed that (a) in the EOs obtained by hydrodistillation, the major components found in the flowers were (*E*)-hex-2-enal (4.0%), hexan-1-ol (4.2%), (*Z*)-hex-2-en-1-ol (6.3%) and palmitic acid (8.3%); (b) in the roots, the major components were α-isocomene (15.4%), α-gurjunene (9.6%), β-isocomene (10.3%), *trans*caryophyllene (10.4%) and palmitic acid (5.3%); (c) the major components of EOs in the leaves were *trans*-caryophyllene (8.7%), germacrene-D (10.2%) and caryophyllene oxide (4.5%).

Bixaceae family

Bixa orellana: *B. orellana*, also known as the 'anatto' or 'achiote' plant, is a small perennial tree, native of South and Central American forests (Lopes *et al.*, 2012). Monzote *et al.* (2014*a*) analysed the EO of the seed of *B. orellana* by gas chromatography-mass spectrometry analysis and reported ishwarane (18.6%), geranylgeraniol (9.1%), and bicyclogermacrene (8.4%) as the major components. This EO was active against *L. amazonensis* and controlled the progression of cutaneous disease in BALB/c mice; at the same time, the CC50 was 7-fold higher for host cells when compared with that for the parasites (Monzote *et al.*, 2014*a*). The EO of *B. orellana* seeds has a complex chemical composition, as its activity could be attributed to geranylgeraniol, which induced mitochondrial alteration, abnormal chromatin condensation in the nucleus, and an increase in superoxide anion production ultimately leading to apoptosis-like cell death (Lopes *et al.*, 2012).

Burseraceae family

Protium heptaphyllum: P. heptaphyllum, commonly called 'almecegueira', is known to produce an amorphous resin which is obtained from the stem; its constituents are compounds such as α- and β-amyrin, taraxastan-3-oxo-20-ol, and sitostenonein (Nogueira *et al.*, 2019). Houel *et al.* (2015) examined whether the antidermatophytic activity of the EO may be an indicator for the discovery of active natural products against *L. amazonensis*. Since *P. heptaphyllum* exhibited a good anti-dermatophytic activity against filamentous fungi and axenic amastigotes (IC_{50} 3.7 μg mL^{-1}), it indicates a correspondence between both the activities. *P. heptaphyllum* is rich in the aromatic monocyclic monoterpene, p-cymene [1-methyl-4-(1-methyl ethyl) benzene], that is the biological precursor of carvacrol (de Cassia da Silveira *et al.*, 2017). The *p*-cymene was also shown to diminish NO production in murine macrophages incubated with lipopolysaccharide (de Santana *et al.*, 2015).

Chenopodiaceae family

Chenopodium ambrosioides: C. ambrosioides is an aromatic and medicinal plant found in the tropics and several regions of America and Africa (Cruz *et al.*, 2007). Monzote *et al.* (2006) observed an intense inhibitory action of the EO against promastigote and amastigote forms of *L. amazonensis*, with IC_{50} values of 3.7 and 4.6 μg mL^{-1}, respectively. Additionally, they noted that BALB/c mice infected and treated with the EO (30 mg kg^{-1}) for 15 days by intraperitoneal route, presented with a reduction in the size of the lesions and suppression of the number of parasites in the infected footpads when compared to the control animals. Monzote *et al.* (2007) confirmed these results; intraperitoneal treatment reduced parasite burden, oral administration delayed infection, and the intralesional route was not valid. Monzote *et al.* (2009) compared the antileishmanial effect of *C. ambrosioides* EO in different doses administered by oral route in BALB/c mice and the conventional drugs, glucantime, amphotericin B and pentamidine, all administered for 15 days. The EO in a 150 mg kg^{-1} dose exhibited better antileishmanial activity and no macroscopic toxic effects. Interestingly, a study demonstrated synergism between *C. ambrosioides* EO with pentamidine against *L. amazonensis* promastigotes (Monzote *et al.*, 2007). The high-resolution gas chromatography-mass spectrometry (HRGC-MS) analysis of this EO showed that its main components were carvacrol (62.36%) and ascaridole (22.54%). *Chenopodium ambrosioides* EO, combined with ascaridole compound, exhibited potent antileishmanial activity against promastigote and amastigote forms. Exploration of its mechanism of action suggests a breakdown of mitochondrial membrane potential and a modification of redox indices (Monzote *et al.*, 2014*b*).

Fabaceae family

Copaifera martii: *Copaifera* are trees found in Latin America and West Africa; they live for about 400 years, reaching between 25 and 40 m in height. The origin of the name seems to have come from the native language of the Tupi Indians 'cupa-yba' which means 'depot tree'. Santos *et al.* (2008) studied eight different kinds of Brazilian *Copaifera* oils for antileishmanial activity and observed activity against promastigote forms of *L. amazonensis* (IC_{50} 5–22 μg mL^{-1}). Oral administration with *C. martii* oil caused a significant reduction in average lesion size in *L. amazonensis* infection (dos Santos *et al.*, 2011). Morphological and ultrastructural analyses demonstrated notable changes in parasite cells treated with this oil. The main ultrastructural effect was mitochondrial swelling. *Copaifera martii* EO led to an increase in plasma membrane permeability and depolarization in the mitochondrial membrane potential in parasite cells. Development of *C. martii* oil formulation as a nanoemulsion in a delivery system led to a reduction in *L. amazonensis* infection levels in macrophage cultures and ultrastructural analyses by SEM revealed that exposure to nanoemulsions induced change in parasite cell shape to oval and retracted flagellae. The treatment of *L. amazonensis*-infected BALB/c mice with nanoemulsions showed significant beneficial effects on lesion size, parasite burden and lesional histopathology (Dhorm Pimentel de Moraes *et al.*, 2018).

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Vouacapoua americana: *V. americana* exhibits slow growth and has potential economic value, occurring in small subpopulations in the French Guiana, Guyana, Peru, Suriname and the Brazilian states of Amapá, Pará, Amazonas and Maranhão. Its wood is widely used in construction and shipbuilding. Moreover, the species grows in areas that undergo strong anthropization and the decline of habitat quality is constant (Brasil, Ministério da Saúde, 2017). *Vouacapoua americana* EO shows activity against *Microsporum gypseum*, *M. canis* and *Trichophyton mentagrophytes*, important dermatophytic fungi. On axenic amastigote forms of *L. amazonensis*, the IC_{50} of the EO was 7.2, with no cytotoxicity in BALB/c mice peritoneal macrophages and VERO cells (Houel *et al.*, 2015).

Pterodon pubescens: The components extraction from *P. pubescens* by 'Supercritical CO_2 extraction' ($scCO_2$), which does not use organic solvents and is environmentally sustainable, detected high geranylgeraniol and 14,15-epoxy-geranylgeraniol content (da Silva Santos *et al.*, 2016). This extract had high inhibitory activity against intracellular amastigotes of *L. amazonensis* (IC_{50} 1.9 $\mu g\,mL^{-1}$), and the effect was likely due to the high geranylgeraniol derivative content of the fluid, which resulted in superoxide anion production, leading to parasite death (Lopes *et al.*, 2012; da Silva Santos *et al.*, 2016).

Lamiaceae family

Tetradenia riparia: *T. riparia* is native to South Africa, where it is one of the most aromatic and popular medicinal plants (Gazim *et al.*, 2014). The EO from *T. riparia* is a complex mixture of terpenoids, including monoterpenes, sesquiterpenes and diterpenes, the most representative class (Gazim *et al.*, 2010, 2014). The most considerable amount of EO in *T. riparia* was found during winter, and the oil content decreased significantly in spring (Gazim *et al.*, 2010). In this context, the EOs of *T. riparia* obtained in spring, summer, autumn and winter showed similar activity against *L. amazonensis*. However, BALB/c mice infected with *L. amazonensis* and treated topically did not present with a reduction in lesion size, but parasite load in the spleen decreased significantly (Cardoso *et al.*, 2015). Demarchi *et al.* (2015) observed that the EO of *T. riparia* is more effective in promoting the death of promastigote and amastigote forms of *L. amazonensis* than an isolated diterpene EO, 6,7-dehydroroyleanone. Additionally, increased inducible nitric oxide synthase (iNOS) mRNA expression or nitrite production by macrophages infected with *L. amazonensis* did not occur after 24 h, suggesting that this EO does not act on parasites through this important elimination pathway. The EO and diterpene derived from *T. riparia* probably promoted the death of *Leishmania* parasites through mitochondrial metabolism pathways, a mechanism of cell death that resembles apoptosis. TEM showed that the EO was able to modify the promastigote ultrastructures, suggesting autophagy demonstrated as chromatin condensation, blebbing, membranous profiles and nuclear fragmentation (Demarchi *et al.*, 2015). However, *T. riparia* EO can modulate an immune response. Demarchi *et al.* (2016) reported that IFN-γ production was inhibited in infected macrophages, and the EO blocked this inhibition. Besides, the EO inhibited some of the most critical cytokines necessary for the establishment of infection, including granulocyte-macrophage colony-stimulating factor, IL-4, IL-10 and TNF-α. The diterpene 6,7-dehydroroyleanone induced a decrease in IL-4 levels and an increase in IL-12 (Terron-Monich *et al.*, 2019). Thus, the EO of *T. riparia* is an agent that can stimulate a protective immune response against intracellular pathogens and inhibit or suppress pathological immune reactions.

Ocimum canum: *Ocimum* species comprise medicinal plants used in traditional medicine for the treatment of microbial diseases, helminthic diseases, inflammation, cardiac diseases, hepatic diseases and metabolic diseases, among many others. Uritu *et al.* (2018) and da Silva *et al.* (2018) identified the chemical constituents of *O. canum*, assessed by gas chromatography-mass spectrometry analyses: thymol (42.15%), p-cymene (21.17%) and γ-terpinene (19.81%) were the major compounds. Antiprotozoal activity of the EO against *L. amazonensis* promastigotes and intracellular amastigotes were high (IC_{50} 17.4 and 13.1 $\mu g\,mL^{-1}$, respectively), with low cytotoxicity against BALB/c peritoneal macrophages (CC_{50} 315.3 $\mu g\,mL^{-1}$). The EO of *O. canum* induced ultrastructural alterations promastigotes of *L. amazonensis*, such as the appearance of autophagosome-like structures, discontinuity of nuclear membrane and exocytic activity by the flagellar pocket, and this exacerbated autophagic response can result in parasite cell death (da Silva *et al.*, 2018).

Ocimum gratissimum: *O. gratissimum* is widely found in several geographical regions in South America and Africa and is used as a medicinal plant with analgesic activity. It contains several proanthocyanidins, which have shown significant antioxidant activity, and tannins, saponins, steroids, alkaloids, terpenoids, flavonoids, phenols and cardiac glycosides (Igbinosa *et al.*, 2013). The eugenol-rich EO of *O. gratissimum* inhibited *L. amazonensis* growth, in promastigote and amastigote forms, with an IC_{50} of 135 and 100 $\mu g\,mL^{-1}$, respectively. IC_{50} of eugenol was 80 $\mu g\,mL^{-1}$ (Ueda-Nakamura *et al.*, 2006). According to these authors, the EO showed no cytotoxic effects against mammalian cells. *Leishmania amazonensis* exposed to the EO underwent considerable ultrastructural alterations, as observed by TEM: the appearance of two or more nuclei or flagella suggesting interference in cell division, internal mitochondrial membrane considerably altered with an increased number of crystals, and the mitochondrial matrix appearing less electron-dense in some amastigotes. NO production by the infected macrophages was increased.

Origanum vulgare: *Origanum* is a genus of herbaceous perennials and shrubs native to Europe, North Africa and much of temperate Asia. The plants have strong aromatic leaves and abundant tubular flowers with long-lasting coloured bracts (Uritu *et al.*, 2018). The people from old Egypt used *Origanum* to disinfect and preserve food (Prerna and Vasudeva, 2015). Teles *et al.* (2019) identified 20 compounds in *O. vulgare* EO and the main compounds were cis-p-menth-2-en-1-ol (33.8%) and linalyl acetate (13.9%). However, antiprotozoal activity on promastigotes was low (IC_{50} 405.5 $\mu g\,mL^{-1}$). According to Sanchez-Suarez *et al.* (2013) *O. vulgare* EO of the Colombian species exhibited activity against promastigotes of *L. panamensis*.

Lauraceae family

Nectandra amazonum, *N. gardneri*, *N. hihua* and *N. megapotamica*: EOs extracted by hydrodistillation from stem bark/leaves of *N. amazonum*, *N. gardneri* and *N. hihua* presented sesquiterpene compounds as the major constituents, while phenylpropanoids were predominant in the EO extracted from *N. megapotamica* (Bosquiroli *et al.*, 2017). EOs of *N. gardneri* and *N. megapotamica* induced a significant increase in NO production by infected macrophages, which may mediate the intracellular death of *L. amazonensis* (Bosquiroli *et al.*, 2017).

Cinnamomum zeylanicum: *C. zeylanicum*, commonly called cinnamon, is used as a seasoning in cooking and is cultivated mainly in countries like India, Sri Lanka and China. Extracts, EOs and cinnamon isolates have applications in food, cosmetics and pesticides due to antimicrobial, antioxidant and antifungal properties. Teles *et al.* (2019) identified 15 compounds in *C. zeylanicum* EO, and the major one was cinnamic aldehyde (46.3%). Its anti-promastigote activity was not considered significant (IC_{50} >500 $\mu g\,mL^{-1}$).

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Cryptocarya aschersoniana: The EO of *C. aschersoniana* obtained by hydrodistillation presented monoterpene hydrocarbons (48.8%), limonene (42.3%), linalool (9.7%) and nerolidol (8.6%) as predominant constituents. Its EO had high activity against *L. amazonensis* promastigote forms (IC_{50} 4.46 $\mu g\,mL^{-1}$); however, it also demonstrated relatively high cytotoxicity against mouse peritoneal macrophages (CC_{50} 7.71 $\mu g\,mL^{-1}$) (Andrade *et al.*, 2018).

Meliaceae family

Carapa guianensis: *C. guianensis* is the Amazon's traditional phytotherapeutic, and its EO is used for its anti-microbial and anti-inflammatory properties for skin diseases. The *C. guianensis* seed EO did not exhibit antileishmanial activity; however, three limonoid-rich EO fractions demonstrated activity against intracellular amastigotes of *L. amazonensis*, which was attributed to the compounds 11β-hydroxygedunin and 6α and 11β-diacetoxygedunin (Oliveira *et al.*, 2018). Dhorm Pimentel de Moraes *et al.* (2018) reported the development of nanoemulsions as a delivery system for *C. guianensis* EO (nanoandi), with toxic activity against promastigotes of *Leishmania* species. Ultrastructural analyses by SEM revealed that exposure to nanoemulsion induced changes in the oval cell shape and flagellae retraction. Interestingly, the *C. guianensis* EO nanoemulsions were effective orally in BALB/c mice infected with *L. amazonensis* (Dhorm Pimentel de Moraes *et al.*, 2018).

Myrtaceae family

Syzygium cumini: *S. cumini* is a large tree, popularly known as 'black plum', 'jambolan', 'jamum' or 'java plum'. The chemical composition and biological potential of the EO extracted from *S. cumini* leaves revealed a high abundance of monoterpenes (87.12%), and the major components were α-pinene (31.85%), (Z)-β-ocimene (28.98%) and (E)-β-ocimene (11.7%) (Dias *et al.*, 2013). This EO showed significant activity against promastigote forms of *L. amazonensis* (IC_{50} 60 $\mu g\,mL^{-1}$). The α-pinene has activity against *L. amazonensis* promastigote forms (IC_{50} 19.7 $\mu g\,mL^{-1}$), axenic forms (IC_{50} 15.6 $\mu g\,mL^{-1}$) and intracellular amastigotes (IC_{50} 16.1 $\mu g\,mL^{-1}$) and was more effective than the EO from *S. cumini* against axenic and intracellular amastigotes (Rodrigues *et al.*, 2015). The EO and α-pinene antileishmanial effects were mediated by immunomodulatory activity, as evidenced by an increase in both phagocytic and lysosomal activities and elevated NO levels induced by these components of *S. cumini* (Rodrigues *et al.*, 2013).

Piperaceae family

Piper rivinoides, *P. mosenii* , *P. cernuum*, *P. arboretum*, *P. aduncum*, *P. gaudichaudianum*, *P. xylosteoides*, *P. mikanianum* and *P. diospyrifolium*: Bernuci *et al.* (2016) evaluated the activity of EOs of nine *Piper* species on *L. amazonensis* promastigotes and found IC_{50} <30 $\mu g\,mL^{-1}$ for EOs of *P. rivinoides*, *P. mosenii* , *P. cernuum*, *P. arboretum* and *P. aduncum*. However, the EOs of *P. gaudichaudianum*, *P. xylosteoides* and *P. mikanianum* showed IC_{50} >100 $\mu g\,mL^{-1}$. *Piper diospyrifolium* and *P. aduncum* alone showed activity against intracellular amastigotes. Analyses of EOs obtained from inflorescences and leaves fresh or dried of *P. clausseniannum* identified sesquiterpenes as the main constituents in the leaves, with a predominance of (E)-nerolidol (up to 83%), while in the EO of inflorescences, monoterpenes were the majority, with predominantly linalool (>50%) (Marques *et al.*, 2010). Interestingly, only the EO from fresh leaves of *P. clausseniannum*, which was rich in (E)-nerolidol, inhibited the *L. amazonensis* growth.

Piper demeraranum, *P. duckei* and *P. hispidum*: *P. demeraranum* and *P. duckei* EOs exhibited biological activity against promastigote and amastigote forms of *L. amazonensis*, *P. duckei* EO being the most active (Moura do Carmo *et al.*, 2012). The main constituents found in *P. demeraranum* EO were limonene (19.3%) and β-elemene (33.1%), and in *P. duckei* EO, the major components were germacrene D (14.7%) and *trans*-caryophyllene (27.1%) (Moura do Carmo *et al.*, 2012). The species *P. hispidum* was identified by Houel *et al.* (2015) as the most promising *Piper* EO (IC_{50} 4.7 $\mu g\,mL^{-1}$), and the most abundant compounds found in this EO were sesquiterpenes, notably curzerene and furanodiene.

The EOs are complex mixtures of compounds, which might possess several activities, that act on multiple targets, and cause death of the parasite by various mechanisms (Salehi *et al.*, 2019). The ultrastructural alterations induced by EOs of *Piper* species on *L. amazonensis* were mitochondrial swelling, intense exocytic activity in the flagellar pocket, and vacuoles in the cytoplasm, suggesting the depletion of ergosterol and alteration of the physical properties of the membranes of the parasites (Vendrametto *et al.*, 2010). Additionally, nerolidol from *P. Aduncum* may lead to parasite death by reduction in cell size, loss of mitochondrial membrane potential, phosphatidylserine exposure and DNA degradation (Ceole *et al.*, 2017).

Plantaginaceae family

Otacanthus azureus: Houel *et al.* (2015) described a high *in vitro* activity (IC_{50} 0.7 $\mu g\,mL^{-1}$) for *O. Azureus* EO, and the value was similar to that of the reference compound, amphotericin B (0.3 $\mu g\,mL^{-1}$). This EO was composed of sesquiterpenes, with the main component being β-copaen-4-α-ol (23%), alongside α-humulene (10.6%), α-copaene (8.8%), myrtenal (5.6%), viridiflorol (5.1%) and trans-pinocarveol (4.3%). Interestingly, none of the main components of *O. azureus* EO have been identified as antileishmanial agents.

Poaceae family

Cymbopogon citratus: *C. citratus* is originally from India, possesses aromatic leaves, and is known as a source of ethnomedicines (Puatanachokchai *et al.*, 2002). Santin *et al.* (2009), through the analysis of a chromatogram obtained by gas chromatography coupled with mass spectrometry, identified the monoterpene, citral, a mixture of the stereoisomers, geranial (42.2%), neral (36.3%) and β-myrcene (13.2%) as the main compound in *C. citratus* EO. In the same study, these authors suggested the activity of the EO on promastigotes, axenic amastigote and intracellular amastigote forms of *L. amazonensis* may be explained by the synergistic effects of various compounds of the EO. The promastigote forms of *L. amazonensis* treated with *C. citratus* EO presented notable morphological and ultrastructural alterations by light microscopy, SEM and TEM (Santin *et al.*, 2009). Houel *et al.* (2015) identified *C. citratus* EO as a potent antidermatophytic agent, and with high antileishmanial activity. However, its EO was considered quite toxic and therefore, is not useful as a medicine.

Rubiaceae family

Mitracarpus frigidus: Chemical analysis of the *M. frigidus* EO obtained by hydrodistillation of the aerial parts of the plant resulted in the identification of 12 known compounds, linalool (29.29%) and eugenol acetate (15.85%) were the major constituents, followed by 5-hydroxy-isobornyl isobutyrate (8.41%), 5-methyl-1-undecene (7.69%) and methyl salicylate (6.55%) (Fabri *et al.*, 2012). This EO exhibited a potent antifungal effect

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against *Cryptoccocus neoformans*, *Candida albicans* and an expressive activity against *L. amazonensis* promastigote forms. The antioxidant activity of the EO investigated through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging was significant (IC_{50} of 38 $\mu g\,mL^{-1}$) (Fabri *et al.*, 2012).

Scrophulariaceae family

Achetaria guianensis: Houel *et al.* (2015) examined whether the antidermatophytic activity of EOs can be used as an indicator for the discovery of active natural products against *L. amazonensis*. They examined seven EOs that exhibited potential antimicrobial obtained from fragrant plant species from French Guiana. *Achetaria guianensis* (EO of leaves and stems) was one of the species studied, but the EO did not show activity against dermatophytic filamentous fungi. The activity of the EO on axenic amastigote forms of *L. amazonensis* was high (6.3 $\mu g\,mL^{-1}$), and cytotoxicity expressed as median toxic dose (TD50) on BALB/c mice peritoneal macrophages and VERO cells were 32.5 and 30.7 $\mu g\,mL^{-1}$, respectively (Houel *et al.*, 2015).

Verbenaceae family

Lippia sidoides: *L. sidoides*, popularly known as 'alecrim pimento', is grown mainly in Northeast Brazil. de Medeiros *et al.* (2011) analysed the chemical composition of *L. sidoides* EO through GC/MS and found the oxygenated monoterpene thymol to be its principal constituent (78.4%). Monoterpene hydrocarbons such as ρ-cymene (6.3%) and other compounds were detected in smaller amounts. Study of the EO from *L. sidoides* and its major compound thymol on *L. amazonensis* showed significant activity against promastigote forms. However, thymol showed toxicity against peritoneal macrophages and low selectivity against the promastigotes, when compared with the crude EO. However, no cytotoxic effect was observed in macrophages treated with the crude EO. Incubation of *L. amazonensis*-infected macrophages with *L. sidoides* EO showed a remarkable reduction in amastigote survival within the macrophages. Significant morphological alterations, such as the accumulation of large lipid droplets in the cytoplasm, disrupted membrane and wrinkled cells, were usually seen in parasites treated with the EO (de Medeiros *et al.*, 2011).

Aloysia gratissima: *A. gratissima* is known as 'Brazil lavender' and is commonly used in Brazilian folk medicine for the treatment of digestive and respiratory diseases. The only study on *A. gratissima* identified in this review was conducted by Garcia *et al.* (2018). These authors detected in *A. gratissima* the monoterpene, 1,8-cineole (17.6%) and the sesquiterpene alcohol, guaiol (10.5%), along with other guaiol isomers (azulene-types structures, 7.3%), hydrocarbons of the germacrene-type sesquiterpenes (>17%), trans-caryophyllene and its oxide (7%) in the EO of leaves. The results showed that the EO killed promastigotes and intracellular amastigotes of *L. amazonensis* at an IC_{50} of 25 and 0.16 $\mu g\,mL^{-1}$, respectively. The EO of *A. gratissima* was safe for macrophages with up to 100 $\mu g\,mL^{-1}$, as evaluated by dehydrogenase activity, membrane integrity and phagocytic capacity of macrophages, and did not induce NO in resting macrophages and inhibited the production of NO in lipopolysaccharide-stimulated macrophages. Ultrastructural analysis suggested that the EO and guaiol act directly on parasites, affecting the kinetoplast, mitochondrial matrix and plasma membrane of promastigotes (Garcia *et al.*, 2018).

Zingiberaceae family

Curcuma longa: Study on *C. longa* EO revealed 17 compounds, turmerone (55.43%), β-turmerone (12.02%) and γ-curcumene (6.96%) being the major compounds. *Curcuma longa* EO has potent antipromastigote activity, which showed a 4.87-fold lower IC_{50} value for intracellular amastigotes, when compared to the IC_{50} for promastigotes (Teles *et al.*, 2019). According to these authors, there was a reduction of infection in BALB/c mice. *Curcuma longa* EO inhibited NO production in peritoneal macrophages, suggesting that there may be other possible mechanisms involved in the intracellular anti-amastigote activity of *C. longa* EO (Teles *et al.*, 2019).

Strengths and limitations of the study

We conducted an extensive search in two databases, which was checked and validated by the researchers in this study, to ensure greater accuracy of the findings. The MeSH terms have been tirelessly revised to provide higher sensitivity to research. Our limitations were the search in only two databases, the setting of English, Portuguese, Spanish and French and a limited search period. Despite the vast heterogeneity of the substances identified in the selected studies, we detected a limited number of publications for each EO, which made it difficult to expand the discussion of the findings. It is important to comment that many of the compounds identified in this review study are potential therapeutic targets for the treatment of *L. amazonensis* infection, as they have low toxicity against host cells and potent antileishmanial action. The risk of bias in the selected *in vivo* and *in vitro* studies was moderate. It is essential to highlight that no study reported allocation sequence generation, allocation concealment mechanism, implementation, outcomes and estimation. Another critical point was that only one study applied the domain blinding of personnel and participants, and the random outcome assessment was performed. These flaws tend to increase the risk of bias in publications of an experimental nature.

Conclusion and future directions

The systematic review of 33 experimental assays showed that EOs and other investigated products possess significantly variable antileishmanial activity against promastigote and amastigote forms of *L. amazonensis*. The *in vivo* studies identified that the route of administration of the EO was important, and the intraperitoneal route is more effective. The discovery of new leishmanicidal bioactive agents is eminent in the face of increased incidence of leishmaniasis and current treatment difficulties. Plants and their secondary compounds, such as EOs, are a promising source for the discovery of new active agents and formulation of prototypes for potential drugs, as revealed by the selected 23 primary studies, which also reported the accurate safety margins of the bioactive agents. Many researchers have been investigating and tracking bioactive agents of new plants, being of paramount relevance for the advancement of these studies to the phase of controlled clinical assays.

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Conflict interest. The authors declare that there is no conflict of interest.

Ethical standards. Not applicable.

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