Anti-protozoal activity of essential oils and their constituents against *Leishmania, Plasmodium* and *Trypanosoma*

Activité anti-protozoaire des huiles essentielles et de leurs constituants contre *Leishmania, Plasmodium* et *Trypanosoma*

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RÉSUMÉ. La découverte de nouveaux composés aux structures innovantes actifs contre *Leishmania, Plasmodium* et *Trypanosoma* est primordiale pour répondre aux limites croissantes (résistance, toxicité, voie d'administration, coût,…) des médicaments actuellement disponibles et à l'absence de vaccins efficaces. Dans cette revue, nous avons répertorié les huiles essentielles et leurs constituants dont l'activité anti protozoaire a été publiée de 2013 à avril 2017. Parmi les 157 huiles essentielles et les 51 composés purs analysés, on observe que certains possèdent un potentiel antiparasitaire intéressant et sélectif *in vitro* voire *in vivo.* Dans certains cas des cibles et/ou modes d'action ont été proposés.

ABSTRACT. Because there is no or only low efficient vaccine available for protozoan diseases and current treatments have serious drawbacks in terms of safety, resistance, cost and administration pathway, the search for new active compounds against *Leishmania, Plasmodium* and *Trypanosoma* is crucial. In this review, we focus on papers related to anti-protozoal activity of essential oils and their components and published from 2013 until April 2017. We show here that among the 157 essential oils and 51 pure compounds analyzed, some can be considered as potential anti-parasitic agents showing selective *in vitro* but also *in vivo* activities with sometimes a proposal of their target and/or mode of action.

MOTS-CLÉS. huiles essentielles, *Leishmania, Trypanosoma, Plasmodium*, cibles antiparasitaires.

KEYWORDS. essential oils, Leishmania, Plasmodium, Trypanosoma, anti-parasitic targets

[CDC, https://www.cdc.gov/index.htm (accessed on Feb 5, 2018) and MEI15]
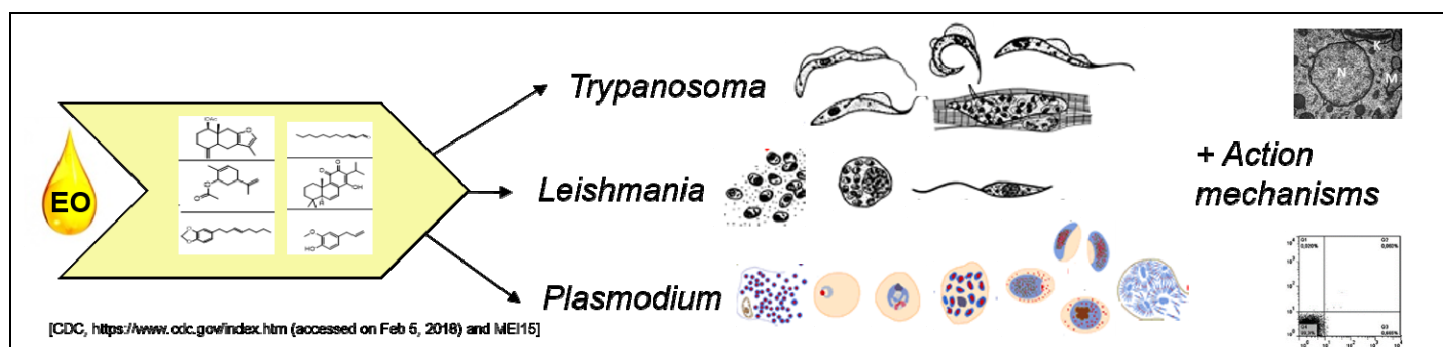
GRAPHICAL ABSTRACT.

Review of *in vitro*, and in some cases *in vivo*, activities of essential oils (EO) and some identified constituents on *Trypanosoma*, *Leishmania* and *Plasmodium* along with their studied modes of action.

1. Introduction

Leishmaniasis, malaria and trypanosomiasis, three common protozoan parasitic diseases, are affecting millions of the world's poorest people. Indeed, one child under five years old is killed by malaria every two minutes and 445,000 deaths were reported worldwide in 2016 with 91% in the African region. For diseases caused by *Trypanosoma*, about 6 to 7 million people are suffering from the Chagas disease and 2184 new cases of sleeping sickness were reported in 2016. In the case of leishmaniasis, over 1 billion people are living in endemic areas at risk of infection and 20,000 – 30,000 deaths are due to this disease annually [1].

Drugs used for treatment of these diseases show several limitations as toxicity, variable efficacy, drug-resistance, requirements for parenteral administration and/or length of treatment. In addition, there is no or only low efficient vaccine, so it is urgent to develop alternative anti-parasitic agents which are safe, effective, affordable and easily administered [2].

Essential oils (EOs), known as volatile oils, are complex mixtures of volatile subtances produced by many aromatic plants [3]. Traditionally, EOs have been used for their antiseptic, analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic properties [3]. Bero *et al.* [4] summarized data about anti-parasitic activities of EOs and their components against *Leishmania, Plasmodium* and *Trypanosoma* until 2012. They highlighted that EOs can be considered as promising bioactive sources.

Continuing that previous review, this article provides an overview of the published data about the anti-parasitic effect of EOs and their constituents from 2013 until April 2017. The search was performed using "Pubmed" and "SciFinder" databases with the keyword "essential oil" combined with *Leishmania*, *Plasmodium* and *Trypanosoma*, respectively.

2. *In vitro* anti-protozoal activity

As mentioned in the Bero's review [4], we considered EOs showing $IC_{50} < 2$ µg/mL (or µM for pure compounds) as strongly active and IC_{50} between 2 and 20 µg/mL (or µM for pure compounds) as moderately active, while those with higher IC_{50} values are less interesting. In some papers, the cytotoxicity was evaluated to calculate the selectivity index (SI) of tested samples. It is recommended for a validated anti-parasitic hit to be at least 10 times more active against parasites than against mammalian cells [5]. In studies on *Leishmania*, toxicity was tested mainly on macrophage cell lines - the direct target of this parasite. For anti-plasmodial and anti-trypanosomal studies, the cytotoxicity was determined either on non-cancer (L6, Vero, CHO, WI38, bovine aortic endothelial cell, red blood cell) or on cancer (KB, HL-60, MCF-7, THP-1, HepG2-A16) cell lines. IC50's are given with their standard deviations, when available.

2.1. *Essential oils*

From 2013 until April 2017, 157 EOs were reported for *in vitro* activity on *Leishmania (L.), Plasmodium (P.)* and/or *Trypanosoma (T.)*. This section only describes EOs with good and moderate activities classified according to their family. Those showing less interesting effects are summarized in Table 1.

2.1.1. *Annonaceae*

The EO extracted by hydrodistillation from dried leaves of *Annona vepretorum* Mart. showed moderate anti-malarial activity against erythrocytic stages of *P. falciparum* and anti-trypanosomal activity against *T. cruzi* epimastigotes and trypomastigotes with IC_{50} of 9.9 ± 0.7, 16.2 ± 1.2, and 11.2 ± 0.5 µg/mL, respectively. The cytotoxicity was evaluated on BALB/c mice peritoneal macrophages and showed $IC_{50} = 39.7 \pm 0.6$ µg/mL [6].

Similarly, EO from dried leaves of *Annona squamosa* L. exhibited IC_{50} value of 14.7 ± 2.9 µg/mL against *P. falciparum*, and IC_{50} values of 14.9 ± 0.7, 12.7 ± 0.5 µg/mL respectively against epimastigote and trypomastigote forms of *T. cruzi*. This EO was more toxic to peritoneal macrophages than the EO extracted from *A. vepretorum* Mart., with an IC_{50} of 28.8 ± 0.9 µg/mL in the same conditions [6].

Hydrodistillated EO of fresh leaves of *Bocageopsis multiflora* (Mart.) R. E. Fries was evaluated on promastigote form of *L. amazonensis* and presented an IC_{50} of 14.6 ± 0.4 µg/mL. The toxicity evaluated on BALB/c mice macrophages showed an IC_{50} = 42.7 µg/mL [7].

The EO extracted from dried leaves of *Xylopia frutescens* Aubl. revealed reasonable trypanocidal action against *T. cruzi* epimastigotes and trypomastigotes with IC_{50} values of 20.2 ± 1.4 and 11.9 ± 0.6 µg/mL, respectively. Likewise, EOs of two specimens of *Xylopia laevigata* (Mart.) R. E. Fries showed IC_{50} values of 22.2 ± 1.7 and 27.7 ± 0.4 µg/mL on epimastigote form, 12.7 ± 1.9 and 13.4 ± 2.1 µg/mL on trypomastigote form of *T. cruzi*. Moreover, all these EOs significantly decreased (*P < 0.05*) the percentage of infected macrophages but also the number of intracellular parasites at the concentration of 10 µg/mL [8].

2.1.2. *Apiaceae*

Dried aerial parts of *Ferula communis* L. were subjected to hydrodistillation for EO collection. This EO was tested on promastigote form of *L. major* and *L. infantum,* and the toxicity was also evaluated on the macrophage cell line Raw 264.7. The sample displayed strong effects on both *Leishmania* species with IC_{50} of 0.11 ± 0.04 and 0.05 ± 0.01 µg/mL respectively. A lower cytotoxicity was reported with a SI of 37.09 compared to *L. major* and 81.60 compared to *L. infantum* [9].

The hydrodistilled EOs of four different parts of *Smyrnium olusatrum* L., fruits, flowers, leaves and roots, presented notable activity against *T. brucei* bloodstream form with IC_{50} values of 1.97 ± 0.06, 3.0 ± 0.4, 3.7 ± 0.5 and 4.0 ± 0.5 µg/mL, respectively. These EOs were selective on parasites as indicated by their SI higher than 23, calculated in comparison to cytotoxicity on mouse BALB/3T3 fibroblasts [10].

2.1.3. *Asteraceae*

The EO of *Artemisia campestris* L. was assayed for anti-leishmanial activity and toxicity *in vitro*. It showed moderate effects against promastigote form of *L. major* and *L. infantum* with IC_{50} values of 4.59 ± 0.23 and 3.24 ± 0.50 µg/mL respectively. The cytotoxicity on the macrophage cell line Raw 264.7 was low, expressed by an IC_{50} value of 80.60 ± 0.30 µg/mL [9].

The *in vitro* anti-leishmanial activity of the EO extracted from *Artemisia absinthium* L. was analyzed on *L. amazonensis*. It demonstrated dose-dependent effects against promastigote and amastigote forms of *L. amazonensis* with IC_{50} of 14.4 ± 3.6 and 13.4 ± 2.4 µg/mL respectively. The cytotoxicity was tested on BALB/c mice peritoneal macrophages and showed an IC_{50} value of 75.1 ± 2.3 µg/mL [11].

The profiles of *in vitro* anti-leishmanial activity and toxicity of the EO extracted from *Artemisia annua* L. fresh leaves were reported. It exhibited notable effects against *L. donovani* promastigotes and intracellular amastigotes with IC_{50} values of 14.63 ± 1.49 and 7.3 ± 1.85 µg/mL, respectively. Interestingly, this EO presented no toxicity not only *in vitro* against mammalian macrophages at 200 µg/mL but also *in vivo* in a BALB/c mice model (up to the highest dose of 200 mg/kg b.w. intraperitoneally) [12].

The EO extracted from dried aerial parts of *Artemisia herba-alba* Asso displayed promising leishmanicidal potential against promastigote form of *L. infantum* and *L. major* with IC_{50} values of

1.22 ± 0.20 and 2.78 ± 0.19 µg/mL, respectively. The cytotoxic effect on macrophage cell line Raw 264.7 was assessed and the IC_{50} was 8.80 ± 0.50 µg/mL [9].

The antiprotozoal effect of the EO extracted from dried leaves of *Artemisia indica* Willd. was screened against *Plasmodium, Trypanosoma* and *Leishmania*. It exhibited moderate activities on blood-stage forms of *P. falciparum* multidrug-resistant strain and bloodstream form of *T. brucei rhodesiense* with IC_{50} values of 9.3 and 2.4 µg/mL respectively. However, this EO was less active on *T. cruzi* intracellular amastigotes and *L. donovani* axenic form. The cytotoxicity on the rat skeletal myoblast cells showed an IC_{50} of 64 µg/mL [13].

The EO extracted from fresh aerial parts of *Mikania micrantha* Kunth was found to exhibit a medium effect against *L. amazonensis* axenic amastigotes with an IC_{50} value of 6.8 µg/mL. SI of 7 and 14 compared to BALB/c mice peritoneal macrophages and Vero cells, respectively, indicated selective action of this EO towards this parasite [14].

The EO of *Vanillosmopsis arborea* (Gardner) Baker (a synonym of *Eremanthus arboreus* (Gardner) MacLeish) inhibited the growth of *L. amazonensis* promastigotes and intracellular amastigotes with IC_{50} values of 7.35 ± 0.05 and 12.58 ± 0.07 µg/mL respectively. The macrophages J774.G8 treated with this EO revealed less toxicity with an IC_{50} of 145 ± 0.023 µg/mL [15].

The EO extracted from *Vernonia polyanthes* Less. (a synonym of *Vernonanthura phosphorica* (Vell.) H.Rob.) showed a moderate effect against promastigote form of *L. infantum* as indicated by an IC_{50} value of 19.4 µg/mL [16].

2.1.4. *Bixaceae*

The EO obtained by hydrodistillation from the dried seeds of *Bixa orellana* L. was evaluated *in vitro* against *L. amazonensis* intracellular amastigote form. A moderate effect was observed (IC_{50} = 8.5 ± 0.8 µg/mL). The IC_{50} on peritoneal macrophages was sevenfold higher than that on parasite [17].

2.1.5. *Burseraceae*

The anti-leishmanial activity of the EO extracted from fresh green fruits of *Protium heptaphyllum* (Aubl.) Marchand was identified on *L. amazonensis* axenic and intracellular amastigotes. It was only moderately active against the axenic form (IC_{50} = 3.7 µg/mL). The weak activity on intracellular form was confirmed with a peritoneal macrophage infection reduction of 59.6% at the concentration of 40 µg/mL. This EO exhibited selective effect on parasite compared to mammalian cells, as its IC_{50} value was 71.2 µg/mL on BALB/c mice peritoneal macrophages and no toxicity was observed on Vero cells at the maximal concentration tested (100 µg/mL) [14].

2.1.6. *Chenopodiaceae*

The data on promising *in vitro* and *in vivo* effects of the EO extracted from *Chenopodium ambrosioides* L. (a synonym of *Dysphania ambrosioides* (L.) Mosyakin & Clemants) against *L. amazonensis* has already been summarized in our previous review [4]. This EO also revealed significant activities against *P. falciparum*, *T. brucei brucei* trypomastigotes, *T. cruzi* and *L. infantum* intracellular amastigotes with IC_{50} values of 0.2 ± 0.2, 0.2 ± 0.07, 1.9 ± 0.3 and 6.4 ± 0.6 µg/mL, respectively. More interestingly, parasiticidal activity of this EO was selective compared to mammalian cells as shown by an IC_{50} value of 58.2 ± 0.05 µg/mL on mouse peritoneal macrophages [18].

2.1.7. *Fabaceae*

The EO extracted from *Vouacapoua americana* Aubl. wood was screened for anti-leishmanial activity against *L. amazonensis* axenic amastigotes and cytotoxicity on BALB/c mice peritoneal

macrophages. Despite the moderate effect observed (IC_{50} value of 7.2 µg/mL), this EO was not further investigated for anti-leishmanial activity because of a SI of 5 compared to mammalian cells [14].

2.1.8. *Geraniaceae*

The EO of *Pelargonium graveolens* L'Hér. aerial parts revealed very interesting anti-leishmanial effects because of low IC_{50} values on promastigote forms of *L. infantum* (0.11 ± 0.06 µg/mL) and *L. major* (0.28 ± 0.08 µg/mL) and an higher IC_{50} value on macrophage cell line Raw 264.7 (6.31 ± 0.17 µg/mL, SI = 57.4 and 22.5 respectively) [9].

2.1.9. *Hypericaceae*

The EO extracted from air-dried aerial parts of *Hypericum scabrum* L. showed a moderate anti-malarial activity against *P. falciparum* chloroquine resistant strain with an IC_{50} value of 15.7 µg/mL. No cytotoxicity was observed on Vero cells up to the highest tested concentration of 47.6 µg/mL [19].

2.1.10. *Lamiaceae*

Volatile oils extracted from fresh flowering aerial parts of three Lamiaceous plants growing in Saudi Arabia were assessed for anti-leishmanial activity against *L. donovani* promastigotes. All samples exhibited reasonable effects with IC_{50} values of 2.3 (*Teucrium polium* L.), 3.7 (*Mentha australis* R. Br.) and 6.1 µg/mL (*Mentha microphylla* K. Koch) [20]. Another EO sample of *Teucrium polium* presented very strong inhibitory activities against the growth of *L. infantum* and *L. major* promastigotes with IC_{50} values of 0.09 ± 0.02 and 0.15 ± 0.09 µg/mL, respectively. An important feature is their selectivity indices compared to macrophage cell line Raw 264.7 of 40.44 (for *L. infantum*) and 24.26 (for *L. major*) [9].

Essid *et al.* also determined notable leishmanicidal action of EOs extracted from dried aerial parts of *Rosmarinus officinalis* L., *Salvia officinalis* L., and *Thymus hirtus* Banks & Sol. (a synonym of *Micromeria graeca* (L.) Benth. ex Rchb.). On *L. infantum* promastigotes, their IC_{50} values were 16.34 ± 0.36, 2.67 ± 0.33, 5.90 ± 0.45 µg/mL respectively and on *L. major* promastigotes, these values were 20.92 ± 0.67, 3.40 ± 0.16, 8.80 ± 0.78 µg/mL respectively. Among these three EOs, the *Salvia officinalis* EO was more toxic on macrophage cell line Raw 264.7 (SI < 10) than the others (SI >10) [9].

The EO of *Mentha crispa* L. (a synonym of *Mentha spicata* L.) demonstrated a dose-dependent effect on the growth of *T. brucei brucei* bloodstream form with an IC_{50} value of 0.33 ± 0.03 µg/mL. This action was selective towards parasite, as shown by SI of 25 compared to HL-60 cells [21].

The EO extracted from leaves of *Tetradenia riparia* (Hochst.) Codd was evaluated for anti-leishmanial activity against *L. amazonensis*. This sample showed promising effects with IC_{50} of 0.5 and 0.03 µg/mL against promastigote and intracellular amastigote forms respectively. Treatment of infected macrophages at the concentration of 0.03 µg/mL reduced the infection index to 54 compared to 114 for untreated group. The cytotoxicity was tested on BALB/c mice macrophages and the IC_{50} value was 0.2 µg/mL, demonstrating the selective effect of this EO towards the parasite [22], [23]. In another assay, *T. riparia* was collected at different seasons. Interestingly, the anti-leishmanial activity against *L. amazonensis* promastigotes was independent from the time of collection (IC_{50} spring = 15.47 ± 4.64 ng/mL, IC_{50} summer = 15.67 ± 1.70 ng/mL, IC_{50} fall = 15.66 ± 2.22 ng/mL and IC_{50} winter = 13.31 ± 0.85 ng/mL). However, the toxicities evaluated on J774.A1 and BABL/c mice peritoneal macrophages were different. The fall EO was the most toxic sample with IC_{50} values of 391.66 ± 17.34 and 65.15 ± 23.20 ng/mL respectively. IC_{50} values of the three other samples, spring, summer and winter, were respectively $1,044.44 \pm 55.55$, $1,476.00 \pm 24.00$ and $1,022.21 \pm 72.85$ ng/mL on J774.A1 cells and 90.94 ± 22.54, 84.37 ± 5.30 and 71.25 ± 31.82 ng/mL on murine macrophages [24].

2.1.11. *Lauraceae*

The EO of *Cinnamomum verum* J.Presl. bark displayed moderate anti-trypanosomal activities against *T. cruzi* epimastigotes and metacyclic trypomastigotes with IC_{50} values of 24.13 ± 1.13 and 5.05 ± 1.03 µg/mL, respectively. The cytotoxicity on Vero cells was also tested and IC_{50} value was 49.4 ± 1.12 µg/mL. On infected Vero cells, 24h incubation with 20 µg/mL showed a 50% reduction of parasite number compared to untreated infected cells [25].

Another EO extracted from aerial parts of a Lauraceae species, *Laurus nobilis* L., presented a moderate effect against promastigote form of *L. infantum* (IC_{50} value of 13.24 ± 0.70 µg/mL). Compared to IC_{50} value of 380.40 ± 0.23 µg/mL against Raw 264.7 macrophage cells, the activity of this EO was selective on parasites [9].

2.1.12. *Meliaceae*

The steam-distillated EO extracted from leaves of *Cedrelopsis grevei* Baill. & Courchet presented a notable inhibitory effect against the growth of chloroquine-resistant *P. falciparum* with an IC_{50} value of 17.5 ± 1 µg/mL [26].

2.1.13. *Myrtaceae*

Two EOs extracted from dried aerial parts of *Eucalyptus globulus* Labill. and *Myrtus communis* L. were tested for anti-leishmanial activity on promastigote form of *L. infantum* and *L. major* and for cytotoxicity on Raw 264.7 macrophages. Both samples demonstrated moderate selective effect towards parasites. IC_{50} values of the *E. globulus* EO against *L. infantum* and *L. major* were 16.28 ± 1.60 and 18.30 ± 1.40 µg/mL, respectively, while these values for *M. communis* were 4.58 ± 0.16 and 6.28 ± 0.52 µg/mL. IC_{50} of these EOs on macrophages were 310.10 ± 0.76 µg/mL for *E. globulus* and 127.60 ± 0.13 µg/mL for *M. communis* [9].

Similarly, the EO of *M. communis* also showed middle effects against *L. tropica* promastigotes and intracellular amastigotes with IC_{50} values of 8.4 ± 0.6 and 11.6 ± 1.2 µg/mL respectively. Cytotoxic effect of this sample against J774 macrophages was low with an IC_{50} of 136.3 ± 7.2 µg/mL [27].

The EO extracted by hydrodistillation from fresh leaves of *Eugenia pitanga* (O.Berg) Nied. revealed a moderate activity against *L. amazonensis* promastigotes with an IC_{50} value of 6.10 ± 1.80 µg/mL [28].

The EO from leaves of *Eugenia uniflora* L. showed IC_{50} values of 11.20 ± 2.17 µg/mL against *T. brucei brucei* bloodstream form, 3.04 ± 0.75 and 1.92 ± 0.8 µg/mL respectively against *L. amazonensis* promastigotes and intracellular amastigotes. This EO showed less toxicity on mammalian cells, expressed by IC_{50} values of 76.40 ± 11.95 µg/mL on the MCF-7 breast cancer cell line and 45.3 ± 2.45 µg/mL on BALB/c mice macrophages [29], [30].

The hydrodistilled EO from fresh leaves of *Plinia cerrocampanensis* Barrie was evaluated for *in vitro* anti-malarial activity on chloroquine-resistant (W2) and chloroquine-sensitive (HB3) strains of *P. falciparum* and for toxicity on Vero cells. It was effective with IC_{50} values of 7.3 and 10.2 µg/mL respectively against both parasite strains. The concentration causing 50% toxicity to mammalian cells was four-fold higher compared to *Plasmodium* ($IC_{50} = 28.6$ µg/mL) [31].

The EO extracted by steam distillation from flower buds of *Syzygium aromaticum* (L.) Merr. & L. M. Perry showed a dose-dependent anti-leishmanial activity with $IC_{50} = 21 \pm 0.16$ and 15.25 ± 0.14 µg/mL respectively against *L. donovani* promastigotes and intracellular amastigotes. This EO revealed no toxicity on murine macrophage cell line Raw 264.7 even at the highest concentration used (200 µg/mL) [32].

2.1.14. *Piperaceae*

The hydrodistillated EO extracted from *Piper aduncum* L. leaves demonstrated reasonable activity against different forms of *T. cruzi* with IC_{50} values = 2.8, 12.1 and 9 µg/mL respectively on cell-derived trypomastigotes (which came from contaminated patients), metacyclic trypomastigotes and amastigotes, however it was inactive on the epimastigote form (IC_{50} = 84.7 µg/mL). Cytotoxicity of this EO was evaluated on Vero cells and showed an IC_{50} = 42.8 µg/mL [33].

The EO of *Piper angustifolium* Lam. (a synonym of *Piper consanguineum* (Kunth) Steud.) leaves displayed a significant effect against *L. infantum* intracellular amastigotes with an IC_{50} value of 1.43 µg/mL. More interestingly, this activity was selective compared to mammalian cells, indicated by SI of 33.72 on fibroblast cells (NIH/3T3) and 22.15 on murine macrophage cells (J774.A1) [34].

Four EOs extracted from fresh leaves of *Piper arboreum* Aubl., *Piper diospyrifolium* Kunth, *Piper mosenii* C. DC. and *Piper rivinoides* Kunth showed moderate effects against *L. amazonensis* promastigotes with IC_{50} values of 15.2 ± 2.4, 13.5 ± 0.4, 17.4 ± 5.0 and 10.9 ± 2.7 µg/mL, respectively. However, all these samples were inactive on the axenic amastigote form. The cytotoxicity tested on BALB/c mice macrophages showed IC_{50} values of 179.1 ± 1.0 and 117 ± 3.0 µg/mL respectively for *P. diospyrifolium* and *P. mosenii* EOs. The two other samples were not toxic on this cell line at the maximal concentration tested (200 µg/mL) [35].

EOs extracted by microwave-assisted hydrodistillation from the aerial parts of six different species of *Piper* grown in Colombia were screened for anti-protozoal activity and cytotoxicity. Two samples of *P. brachypodon* (Benth.) C. DC., collected in two different locations (signed as EO10 and EO11), exhibited the most promising effects against *T. cruzi* epimastigotes with IC_{50} values of 0.34 and 1.74 µg/mL respectively. However, they showed less interesting effects against the amastigote form (IC_{50} > 100 µg/mL for EO10 and IC_{50} = 22.72 µg/mL for EO11). Both of them were selective on epimastigotes parasites as shown by IC_{50} values of 30.54 and 52.55 µg/mL on Vero cells, and 66.31 and 62.82 µg/mL on THP-1 cells (EO10 and EO11 respectively). Other EOs extracted from *P. bogotense* C. DC. (EO3), *P. marginatum* Jacq. (EO4), *P. divaricatum* G. Mey. (EO5), *P. septuplinervium* (Miq.) C. DC. (EO8), and *P. lanceifolium* Kunth (EO9) revealed moderate anti-trypanosomal activities against *T. cruzi* epimastigotes with IC_{50} = 10.09, 16.15, 13.05, 13.98 and 7.48 µg/mL respectively, however all of them were inactive on the amastigote form. EO3, EO4 and EO5 were not toxic against THP-1 cells at the highest concentration tested (100 µg/mL), while their IC_{50} values on Vero cells were 90.08, 40.21 and 89.75 µg/mL respectively. EO8 and EO9 showed similar effects against both mammalian cell lines with IC_{50} = 42.67 and 46.03 µg/mL (Vero), 48.81 and 55.70 µg/mL (THP-1), respectively [36].

EOs from fresh aerial parts of *Piper claussenianum* (Miq.) C. DC. and *Piper lucaeanum* var. *grandifolium* Yunk (from Brazil according to IPNI identification) demonstrated reasonable anti-plasmodial activities against *P. falciparum* chloroquine-resistant strain (W2) with IC_{50} values of 7.9 and 2.65 µg/mL respectively. The *P. lucaeanum* EO was further analyzed for cytotoxicity on HepG2 cells and it showed selectivity towards parasites as evidenced by a SI of 11.94 [37].

On *L. amazonensis*, the EO of *Piper hispidum* Sw. displayed notable effects against both axenic and intracellular amastigote forms with IC_{50} values respectively of 3.4 and 4.7 µg/mL. At the highest concentration tested (20 µg/mL), this EO decreased by 97.5% the infection index compared to untreated cells in *Leishmania* infected macrophages assay. With an IC_{50} value of 35.5 µg/mL against BALB/c mice peritoneal macrophages, this sample showed a quite selective activity on parasite [14].

2.1.15. *Plantaginaceae*

The EO extracted from fresh aerial parts of *Otacanthus azureus* (Linden) Ronse (a synonym of *Achetaria azurea* (Linden) V. C. Souza) showed strong to moderate inhibitory effects on the growth of

L. amazonensis axenic and intracellular amastigotes with IC_{50} values of 0.7 and 16.1 μg/mL, respectively. The cytotoxicity was evaluated against BALB/c mice peritoneal macrophages and the IC_{50} value was 35.5 μg/mL [14].

2.1.16. Poaceae

The EO extracted from fresh leaves of *Cymbopogon citratus* (DC.) Stapf displayed anti-protozoal activities against *Leishmania, Trypanosoma* and *Plasmodium.* The sample collected in French Guiana was effective against *L. amazonensis* axenic amastigotes with IC_{50} = 5.3 μg/mL but IC_{50} on BALB/c mice peritoneal macrophages and Vero cells were 25.2 and 10.7 μg/mL, respectively [14]. The sample collected in Benin showed a strong effect against the bloodstream form of *T. brucei brucei* with an IC_{50} value of 1.83 ± 0.13 μg/mL. Cytotoxicity of this EO was evaluated on Chinese Hamster Ovary cells (CHO) and human normal fibroblast cells (WI38) and IC_{50} values were respectively 10.63 ± 0.72 and 39.77 ± 3.31 μg/mL [38]. On *P. falciparum, C. citratus* EO collected in Cameroon showed a notable effect against the chloroquine-resistant strain with IC_{50} = 4.2 ± 0.5 μg/mL [39], unlike the Benin EO on a chloroquine-sensitive strain (IC_{50} = 47.97 ± 13.09 μg/mL) [38].

Also collected from Benin, the EO of *Cymbopogon giganteus* Chiov. demonstrated significant to middle effects against *T. brucei brucei* bloodstream form and *P. falciparum* chloroquine-sensitive strain with IC_{50} values of 0.25 ± 0.11 and 11.22 ± 5.35 μg/mL respectively. Notably, this EO showed no toxicity on both mammalian cell lines (CHO and WI38) even at the maximal concentration of 50 μg/mL [38].

EOs extracted from fresh leaves of two other *Cymbopogon* species collected from Benin, *C. schoenanthus* (L.) Spreng. and *C. nardus* (L.) Rendle, also showed anti-trypanosomal activities against *T. brucei brucei* bloodstream form with IC_{50} = 2.10 ± 0.89 and 5.71 ± 1.40 μg/mL respectively. Interestingly, both EOs did not reveal any toxicity on mammalian cells (CHO and WI38) at the concentration of 50 μg/mL [38]. On this *Trypanosoma* species, the EO extracted from fresh whole plants of *C. nardus* (L.) Rendle in Malaysia demonstrated a strong activity with IC_{50} = 0.31 ± 0.03 μg/mL and was not toxic against Vero cells at the highest concentration tested (100 μg/mL) [40].

2.1.17. Ranunculaceae

EOs of *Nigella sativa* L. showed selective anti-leishmanial activity compared to mammalian cells. The sample extracted from dried aerial parts, collected in Tunisia, displayed effects against promastigote forms of *L. infantum* and *L. major* with IC_{50} values of 10.68 ± 1.53 and 13.25 ± 1.14 μg/mL respectively. The toxicity of this EO on macrophage cell line Raw 164.7 was very low with IC_{50} = 220.78 ± 0.90 μg/mL [9]. The sample extracted from seeds collected in Iran, also displayed a similar effect against *L. infantum* promastigotes with IC_{50} = 11.7 ± 1.15 μg/mL. Moreover, this EO showed activity against *L. tropica* with IC_{50} = 9.3 ± 2.08 μg/mL. On intracellular amastigote forms of *L. infantum* and *L. tropica*, IC_{50} values were 26.3 ± 2.0 and 21.4 ± 2.15 μg/mL respectively. Promastigotes treatment with 5 μg/mL EO reduced the infection of murine macrophages to 27.3 ± 1.15% (*L. tropica*) and 33.6 ± 1.15% (*L. infantum*) compared to 78.3 ± 2.52% and 81.3 ± 3.51% on non-treated promastigotes. IC_{50} of the seeds' EO on murine macrophage cells was 444.3 ± 4.1 μg/mL [41].

2.1.18. Rutaceae

Two EOs extracted by hydrodistillation from fruits of two Rutaceae species, *Citrus aurantium* L. and *Swinglea glutinosa* (Blanco) Merr., indicated promising activity against *T. cruzi* epimastigotes with less than 30% of viable parasites at the lowest tested concentration (6.25 μg/mL) [42].

2.1.19. *Scrophulariaceae*

The EO from fresh parts of *Achetaria guianensis* Pennell showed anti-leishmanial activity with an IC_{50} value of 6.3 µg/mL against axenic amastigote form of *L. amazonensis*. The cytotoxicity was evaluated on BALB/c mice peritoneal macrophages (IC_{50} = 32.5 µg/mL) and on Vero cells (IC_{50} = 30.7 µg/mL) [14].

2.1.20. *Verbenaceae*

The trypanocidal action of EO extracted from *Lippia pedunculosa* Hayek was evaluated against *T. cruzi* epimastigotes and trypomastigotes and showed IC_{50} values of 15.1 ± 2.4 and 11.3 ± 0.3 µg/mL respectively [43].

As mentioned, all EOs with low to no anti-parasitic activities are reported in the table below.

Family	Plants	Leishmania		Plasmodium		Trypanosoma		Ref.*
		Species - form	IC$_{50}$ (µg/mL)	Species - strain	IC$_{50}$ (µg/mL)	Species - form	IC$_{50}$ (µg/mL)	
Anacardiaceae	*Mangifera indica* L. var. *Espada*	*amazonensis* - proma.	23.0 ± 2.7					**[44]**
	Mangifera indica L. var. *Rosa*	*amazonensis* - proma.	39.1 ± 5.6					**[44]**
	Myracrodruon urundeuva (Engl.) Fr. All (considered as *M. urundeuva* Allemaõ)	*amazonensis* - axe. ama. - proma. - intra. ama.	104.6 ± 11.82 205 ± 13.4 44.5 ± 4.37					**[45]**
	Pistacia vera L.	*tropica* - intra. ama.	21.3 ± 2.1					**[46]**
Annonaceae	*Annona pickelii* (Diels) H.Rainer					*cruzi* - epima.	27.2 ± 1.4	**[47]**
	Annona salzmannii A.DC.					*cruzi* - epima.	89.7 ± 2.4	**[47]**
	Cananga odorata (Lam.) Hook.f. & Thomson	*amazonensis* - proma.	> 500					[48]
	Xylopia laevigata (Mart.) R.E.Fr.					*cruzi* - epima.	93.9 ± 2.6	**[49]**
Apiaceae	*Coriandrum sativum* L.	*donovani* - proma.	26.58 ± 6.11					**[50]**
		major - proma.	> 640					[51]
		braziliensis - proma.	> 640					[51]
		guyanensis - proma.	> 640					[51]
		panamensis - proma.	427.95 ± 118.4					[51]
	Ferula galbaniflua Boiss. & Buhse	*amazonensis* - proma.	95.7 ± 1.82					**[48]**
	Foeniculum officinale All (synonym of *F. vulgare* Mill.)	*amazonensis* - proma.	328.28 ± 6.80					[48]
	Pleurospermum amabile W. G. Craib & W. W. Sm			*falciparum* - multidrug resistant	72.3			**[52]**
				falciparum - chloroquine and antifolate sensitive	79.0			**[52]**
Asteraceae	*Artemisia absinthium* L.					*cruzi* - epima.	144.6	**[53]**
	Artemisia campestris L.	*infantum* - proma.	44					**[54]**
	Artemisia indica Willd.	*donovani* - axe. ama.	24.6			*cruzi* - intra. ama.	51.9	**[13]**
	Artemisia herba-alba Asso.	*infantum* - proma.	68					**[54]**
	Erigeron floribundus (Kunth) Sch.Bip					*brucei* - BSF	33.5 ± 2.7	**[55]**
	Matricaria chamomilla L.	*amazonensis* - proma.	60.16 ± 4.24					**[48]**
	Pulicaria gnaphalodes (Vent.) Boiss.	*major* - proma.	270 (nL/mL)					**[56]**
	Vernonanthura brasiliana (L.) H.Rob. (cited as *Vernonia brasiliana* (Less) Druce) - leaves	*amazonensis* - proma.	213 ± 41			*cruzi* - trypoma.	72 ± 50	**[57]**

Family	Plant species	Leishmania	IC	P. falciparum	IC	T. cruzi	IC	Ref
	Vernonanthura brasiliana (L.) H.Rob. - flowers	*amazonensis* - proma.	112 ± 16			*cruzi* - trypoma.	88 ± 70	[57]
	Vernonanthura brasiliana (L.) H.Rob. - roots	*amazonensis* - proma.	109 ± 12			*cruzi* - trypoma.	70 ± 12	[57]
Boraginaceae	*Cordia verbenaceae* A. DC. (synonym of *Cordia curassavica* (Jacq.) Roem. & Schult.)	*amazonensis* - proma.	64.75 ± 2.04					[48]
	Varronia schomburgkii (DC.) Borhidi (synonym of *Cordia schomburgkii* A. DC.)	*Guyanensis*	> 50					[58]
Canellaceae	*Cinnamodendron dinisii* Schwacke	*amazonensis* - proma.	54.05 ± 4.88					[48]
						cruzi - epima.	282.93	[59]
Cannabaceae	*Cannabis sativa* L	*donovani* - proma.	> 40					[60]
Euphorbia-ceae	*Croton cajucara* Benth.	*chagasi* - proma.	66.7					[61]
Fabaceae	*Myrocarpus frondosus* Allemao					*cruzi* - epima	60.87 ± 1.13	[25]
	Myroxylon peruiferum L.f.	*amazonensis* - proma.	162.25 ± 1.57					[48]
Geraniaceae	*Pelargonium graveolens* L'Hér.	*amazonensis* - proma.	363.71 ± 6.77					[48]
Hypericaceae	*Hypericum scabrum* L.			*falciparum* - CQS	28.8			[19]
Lamiaceae	*Lavandula officinalis* Chaix (synonym of *Lavandula angustifolia* Mill.)	*amazonensis* - proma.	> 500					[48]
	Melissa officinalis L.	*amazonensis* - proma.	132.02 ± 3.14					[48]
	Mentha australis R. Br.			*falciparum* - CQS / - CQR	> 20 / > 20			[20]
	Mentha microphylla K. Koch (synonym of *Mentha spicata* subsp. *condensate* (Briq.) Greuter & Burdet)			*falciparum* - CQS / - CQR	> 20 / > 20			[20]
	Mentha x piperita L. and *Mentha pulegium* L. (50/50)	*major* - proma.	> 640					[51]
		braziliensis - proma.	> 640					[51]
		guyanensis - proma.	> 640					[51]
		panamensis - proma.	> 640					[51]
	Ocimum basilicum L.	*major* - proma.	> 640	*falciparum* - CQR	21.0 ± 4.6			[51], [39]
		braziliensis - proma.	> 640					[51]
		guyanensis - proma.	315.55 ± 90.86					[51]
		panamensis - proma.	251.59 ± 64.18					[51]
	Ocimum canum Sims (synonym of			*falciparum* - CQR	20.6 ± 3.4			[39]

Family	Species						Ref.
	Ocimum americanum L.)						
	Ocimum gratissimum L. - full-flowering			*falciparum* - CQS	49.29 ± 12.35	*brucei brucei* - BSF	27.23 ± 3.74	[62]
	Ocimum gratissimum L. - pre-fowering			*falciparum* - CQS	55.06 ± 14.68	*brucei brucei* - BSF	69.59 ± 7.33	[62]
	Origanum vulgare L.	*major* - proma.	171.8 ± 20.64				[51]
		braziliensis - proma.	204.36 ± 21.56				[51]
		guyanensis - proma.	> 640				[51]
		panamensis - proma.	42.23 ± 2.04				[51]
	Rosmarinus officinalis L.	*major* - proma.	> 640		*cruzi* - epima.	> 300	[51], [25]
		braziliensis - proma.	> 640				[51]
		guyanensis - proma.	> 640				[51]
		panamensis - proma.	> 640				[51]
	Salvia sclarea L.	*amazonensis* - proma.	325.92 ± 8.58				[48]
	Teucrium polium L.			*falciparum* - CQS - CQR	> 20 > 20			[20]
	Thymus capitellatus Hoffmanns. & Link	*infantum* - proma.	37				[63]
		tropica - proma.	35				[63]
		major - proma.	62				[63]
	Thymus syriacus Boiss.	*tropica* - proma.	101.08				[64]
	Thymus vulgaris L.	*major* - proma.	> 640				[51]
		braziliensis - proma.	> 640				[51]
		guyanensis - proma.	> 640				[51]
		panamensis - proma.	402.23 ± 82.90				[51]
	Vitex agnus-castus L.	*tropica* - proma.	211.62				[64]
	Zataria multiflora Boiss	*tropica* - proma - intra. ama.	(nL/mL) 3200 ± 150 8300 ± 600				[65]
Lauraceae	*Cinnamomum camphora* (L.) J. Presl.	*amazonensis* - proma.	> 500				[48]
	Laurus nobilis L.	*major* - proma.	24.36 ± 1.18				[9]
	Litsea cubeba (Lour.) Pers.	*amazonensis* - proma.	> 500				[48]
Myrtaceae	*Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson				*cruzi* - epima.	> 300	[25]
	Eucalyptus globulus Labill.				*cruzi* - epima.	> 300	[25]
	Eugenia uniflora L.				*cruzi* - epima.	70 ± 1.04	[25]
	Syzygium cumini (L.) Skeels	*amazonensis* - axe. ama. - proma. - intra. ama.	43.9 60 38.1				[66], [67]
Piperaceae	*Manekia obtusa* (Miq.) T.Arias,	*amazonensis* - proma.	173.5				[68]

	Species						Ref.
	Callejas & Bornst.	*chagasi* - proma.	> 500				[68]
	Piper aduncum L.	*amazonensis* - axe. ama. - proma.	36.2 ± 2.9 25.9 ± 1.3				[35]
		braziliensis - proma.	77.9				[69]
	Piper auritum Kunth	*infantum* - proma. - intra. ama.	> 100 > 100		*cruzi* - epima. - intra. ama.	> 100 > 100	[36]
	Piper bogotense C. DC.	*infantum* - proma. - intra. ama.	> 100 > 100				[36]
	Piper brachypodon (Benth.) C. DC. - two batches	*infantum* - proma. - intra. ama.	23.43/23.68 > 100/>100				[36]
	Piper cf. brachypodon var. *hirsuticaule* Yunck (according to IPNI)	*infantum* - proma. - intra. ama.	93.6 > 100		*cruzi* - epima. - intra. ama.	32.49 > 100	[36]
	Piper bredemeyeri J. Jacq.	*infantum* - proma. - intra. ama.	> 100 > 100		*cruzi* - epima. - intra. ama.	> 100 > 100	[36]
	Piper cernuum Vell.	*amazonensis* - axe. ama. - proma.	> 200 27.1 ± 0.9				[35]
	Piper cubeba L.f. (unresolved name)	*amazonensis* - proma.	326.5		*cruzi* - trypoma. - intra. ama.	45.5 53.2	[70]
	Piper cf. divaricatum (supposed to refer to G.Mey.)	*infantum* - proma. - intra. ama.	73.29 > 100				[36]
	Piper gaudichaudianum (Kunth) Kunth ex Steud.	*amazonensis* - proma.	93.5 ± 1.6				[35]
	Piper lanceifolium Kunth	*infantum* - proma. - intra. ama.	37.81 > 100				[36]
	Piper marginatum Jacq.	*infantum* - proma. - intra. ama.	88.7 > 100				[36]
	Piper mikanianum (Kunth) Steud.	*amazonensis* - proma.	> 100				[35]
	Piper obrutum Trel. & Yunck.	*infantum* - proma. - intra. ama.	35.87 89.02		*cruzi* - epima. - intra. ama.	28.28 > 100	[36]
	Piper septuplinervium (Miq.) C. DC.	*infantum* - proma. - intra. ama.	30.05 64.8				[36]
	Piper xylosteoides (Kunth) Steud.	*amazonensis* - proma.	> 100				[35]
Poaceae	*Cymbopogon citratus* (DC.) Stapf.	*major* - proma.	194.05 ± 29.20	*falciparum* - CQS	47.97 ± 13.09		[51], [38]
	Cymbopogon citratus (DC.) Stapf.	*braziliensis* - proma.	160.06 ± 43.49				[51]
		guyanensis - proma.	149.1 ± 6.22				[51]
		panamensis - proma.	180.83 ± 82.24				[51]
	Cymbopogon nardus (L.) Rendle			*falciparum* - CQS	52.61 ± 4.79	*cruzi* - epima. 94 ± 1.14	[25], [38]
	Cymbopogon schoenanthus (L.) Spreng.			*falciparum* - CQS	43.15 ± 13.19		[38]
	Citrus limon (L.)	*major*				*cruzi*	[51],

Family	Species					
Rutaceae	Burm. f. (according to IPNI)	- proma.	> 640	- epima.	107.14 ± 1.03	[25]
		braziliensis - proma.	> 640	*brucei brucei* - BSF	60.90 ± 0.91	**[71]**, [51]
		guyanensis - proma.	231.4 ± 42.43			[51]
		panamensis - proma.	> 640			[51]
	Citrus sinensis (L.) Osbeck	*major* - proma.	> 640			[51]
		braziliensis - proma.	> 640			[51]
		guyanensis - proma.	> 640			[51]
		panamensis - proma.	> 640			[51]
Siparunaceae	*Siparuna guianensis* Aubl.	*amazonensis* - proma.	48.55 ± 3.64	*cruzi* - epima.	209.30	**[59]**, **[48]**
Verbenaceae	*Lantana camara* L.	*braziliensis* - proma.	72.31 ± 0.89	*cruzi* - epima.	201.94 ± 1.2	**[72]**
	Lippia gracilis Schauer (contains 61.84% thymol)	*chagasi* - proma.	86.32			**[73]**
	Lippia gracilis Schauer (contains 61.84% carvacrol)	*chagasi* - proma.	77.26			**[73]**
	Nashia inaguensis Millsp.	*guyanensis* - proma.	511.3			**[74]**
Zingiberaceae	*Elettaria cardamomum* (L.) Maton	*amazonensis* - proma.	> 500			[48]
	Zingiber officinale Roscoe	*major* - proma.	303.0 ± 107.48			[51]
		braziliensis - proma.	124.94 ± 52.98			[51]
		guyanensis - proma.	256.95 ± 75.17			[51]
		panamensis - proma.	154.83 ± 23.86			[51]
Zygophylla-ceae	*Bulnesia sarmientoi* Lorentz ex Griseb	*amazonensis* - proma.	85.56 ± 3.38			**[48]**

proma.: promastigote
axe. ama.: axenic amastigote
intra. ama.: intracellular amastigote

CQS: chloroquine-sensitive
CQR: chloroquine-resistant

BSF: bloodstream form
epima.: epimastigote
trypoma.: trypomastigote

*: For references in bold, EO chemical composition was analyzed

Table 1. *In vitro anti-protozoal activity of EOs showing IC$_{50}$ > 20 μg/mL*

2.2. Essential oil components

In the period covered by this review, 51 pure compounds contained in EOs were analyzed for anti-protozoal activities against *Leishmania, Plasmodium* and *Trypanosoma* (Table 2). However no compounds revealed interesting anti-plasmodial activity (IC$_{50}$ ≤ 20 μM). Compounds possessing good and moderate *in vitro* anti-leishmanial and/or -trypanosomal effects are cited below.

2.2.1. Anti-leishmanial activity

Ascaridole, carvacrol, and caryophyllene oxide, major compounds of the EO extracted from *Chenopodium ambrosioides* L. (synonym of *Dysphania ambrosioides* (L.) Mosyakin & Clemants), were analyzed for anti-leishmanial activity against *L. amazonensis* promastigotes and intracellular amastigotes. Among them, ascaridole was the most effective compound with IC$_{50}$ values of 0.59 ± 0.01 and 1.78 ± 0.05 μM respectively, followed by caryophyllene oxide with IC$_{50}$ values of 22.24 ± 2.3 and 19.97 ± 0.4 μM while carvacrol was inactive, but demonstrated anti-leishmanial activity against promastigote form of *L. chagasi* (IC$_{50}$ value of 15.31 μM). However, all of them displayed cytotoxicity

against mouse peritoneal macrophages as shown by SI of 4, 2 and 1, respectively, compared to intracellular amastigotes [18], [73].

α-Bisabolol showed a middle effect against *L. amazonensis* intracellular amastigotes after 24 hours of incubation (IC_{50} = 18.66 ± 0.07 μM). However, this compound showed lower IC_{50} values (> 20 μM) on promastigotes and on both forms in another study. The cytotoxicity evaluation on J774.G8 macrophages showed IC_{50} from 66.65 ± 0.12 to 451.50 ± 0.025 μM [75], [15].

β-Caryophyllene displayed notable anti-leishmanial activity in the study of Essid *et al.* against promastigote forms of *L. infantum* and *L. major* with IC_{50} values of 5.19 ± 0.37 and 6.51 ± 0.52 μM respectively. With an IC_{50} value of 108.00 ± 0.33 μM on macrophage cell line Raw 264.7, this compound showed selective effect towards the parasite [9]. However in the study of Leal *et al.*, this compound was inactive (IC_{50} > 100 μM) on *L. infantum* promastigotes and intracellular amastigotes [36].

Two aldehydes, (*E*)-2-decenal and (*E*)-2-undecenal, known as major compounds in the *Coriandrum sativum* L. EO, were analyzed for *in vitro* anti-leishmanial activity against *L. donovani*. They displayed moderate activity against axenic amastigotes (IC_{50} = 16.01 ± 0.25 and 7.43 ± 0.11 μM respectively) and only (*E*)-2-undecenal showed an effect against promastigotes (IC_{50} = 16.70 ± 0.21 μM). Low or no activity was observed on intracellular amastigotes [50].

6,7-Dehydroroyleanone was isolated from the *Tetradenia riparia* (Hochst.) Codd EO and studied for anti-leishmanial activity against *L. amazonensis* promastigotes. Unfortunately, this compound was effective against parasites with IC_{50} = 7.79 μM but more toxic on BALB/c mice peritoneal macrophages with an IC_{50} value of 1.69 μM [22].

7-Hydroxycalamenene isolated from the EO of *Croton cajucara* Benth. exhibited a notable effect against *L. chagasi* promastigotes with an IC_{50} value of 6.27 μM. Interestingly, this compound did not show toxicity on peritoneal mouse macrophages at the highest concentration tested (2290.11 μM) [61].

Thymoquinone, the major component of the *Nigella sativa* L. EO, demonstrated interesting anti-leishmanial activity in the study of Mahmoudvand *et al.* against *L. tropica* and *L. infantum*. It showed IC_{50} values of 7.06 ± 0.05 and 8.95 ± 0.05 μM on promastigotes and 12.79 ± 0.05 and 15.83 ± 0.1 μM on intracellular amastigotes respectively. Pre-treatment of both species promastigotes at 6.09 μM reduced the macrophages infection percentage to 13% and 16.3% respectively, compared to 78.3% and 81.3% for non-treated promastigotes. An important feature of this compound was its selective effect compared to murine macrophage cells (IC_{50} value of 236.59 μM) [41].

A monoterpenic alcohol, (S)-cis-verbenol, showed a moderate effect on *L. brasiliensis* promastigote form with IC_{50} of 13.79 μM. Compared to its IC_{50} value of 7101 μM on human fibroblast cells, this compound exhibited a selective effect on this promastigote species [76].
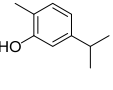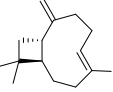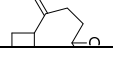
2.2.2. Anti-trypanosomal activity
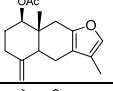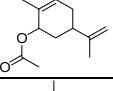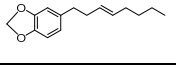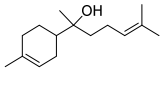
β-Caryophyllene, 1,8-cineole and α-pinene displayed moderate anti-leishmanial activities against *T. cruzi* epimastigotes with IC_{50} values of 14.14, 4.08 and 20.11 μM respectively. However, on intracellular amastigotes, only α-pinene was effective with IC_{50} = 14.09 μM. The cytotoxicity analyzed on Vero cells showed IC_{50} values of 63.27 μM, 411.60 μM and 84.92 μM, respectively [36].
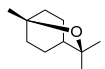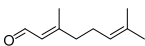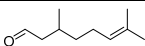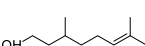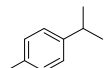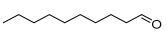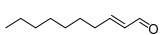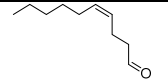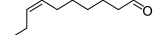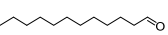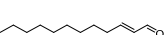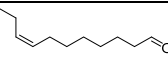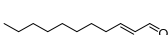
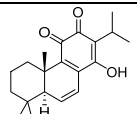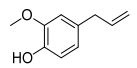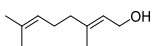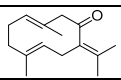Citronellal and myrcene were analyzed for anti-trypanosomal activities against *T. brucei brucei* bloodstream form and for cytotoxicity on human normal fibroblast cells (WI38) and Chinese Hamster Ovary cells (CHO). Medium effects were observed on parasites with IC_{50} = 17.89 ± 1.55 and 16.44 ± 0.27 μM respectively. These compounds did not show any toxicity on mammalian cells at the highest concentration tested (324.15 μM for citronellal and 367.00 μM for myrcene) [38].
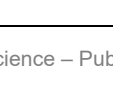
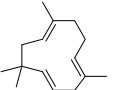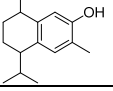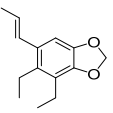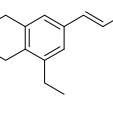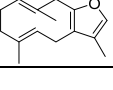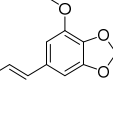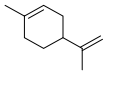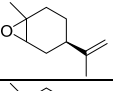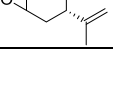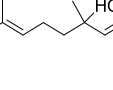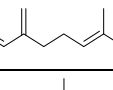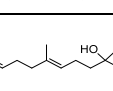Isofuranodiene, identified in the EO of *Smyrnium olusatrum* L., was assayed for anti-trypanosomal activity against bloodstream form of *T. brucei brucei* and for toxicity on mouse BALB/c fibroblasts. With IC_{50} values of 3.0 ± 0.8 µM on the parasite and 91 ± 12 µM on mammalian cells, this compound could be a potential anti-trypanosomal agent [10].
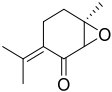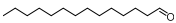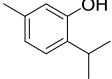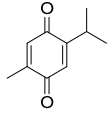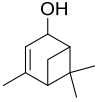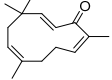
Linalool demonstrated a strong effect against *T. cruzi* cell-derived trypomastigotes with an IC_{50} value of 2.01 µM. However this activity was not selective indicated by a SI = 2.7 compared to Vero cells [33].

Four p-menthane-type monoterpenes, rotundifolone, (+)-limonene epoxide, (-)-limonene epoxide, (-)-perillyl aldehyde, were tested against bloodstream form of *T. brucei brucei*. Rotundifolone displayed the highest effect with $IC_{50} = 1.93$ µM, followed by (-)-perillyl aldehyde, (-)-limonene epoxide and (+)-limonene epoxide with IC_{50} values of 2.06, 19.3 and 19.9 µM respectively. All these compounds showed selective activity (SI > 10) compared to human myeloid leukaemia HL-60 cells [21].

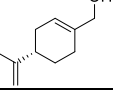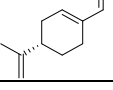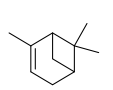| Compounds			*Leishmania*		*Plasmodium*		*Trypanosoma*		Ref.
Name	Structure	Chemical class	Species - form	IC_{50} (µM)	Species - strain	IC_{50} (µM)	Species - form	IC_{50} (µM)	
β-acetoxy-furanoeudesm-4(15)-ene		sesquiterpene					*brucei brucei* - BSF	26 ± 3	[10]
6-acetoxy-*p*-mentha-1,8-diene		monoterpene					*brucei brucei* - BSF	148.35 ± 2.91	[38]
ascaridole		monoterpene	*amazonensis* - proma. - ama.	0.59 ± 0.01 1.78 ± 0.05					[18], [77]
5-[(3*E*)-oct-3-en-1-il]-1,3-benzodioxole			*infantum* - proma. - ama.	82.5 ± 5.2 51.6 ± 4.6			*cruzi* - trypoma. - ama.	67.3 ± 2.6 32.6 ± 1.5	[78]
α-bisabolol		sesquiterpene	*amazonensis* - proma. - intra. ama.	22.26 ± 0.05 48.12 ± 0.09					[15]
			amazonensis - proma. - intra. ama	36.29 ± 0.09 18.66 ± 0.07					[75]
borneol		monoterpene	*infantum* - proma.	> 2593.19					[63]
			tropica - proma.	> 2593.19					[63]
			major - proma.	> 2593.19					[63]
camphor		monoterpene	*infantum* - proma.	36.46 ± 1.27					[9]
			major - proma.	51.90 ± 0.42					[9]
carvacrol		monoterpene	*infantum* - proma.	48.93 ± 1.78					[9]
			major - proma.	60.91 ± 0.12					[9]
			chagasi - proma.	15.31					[73]
			amazonensis - proma. - ama.	101.85 ± 4.6 90.53 ± 1.8					[18], [77]
β-caryophyllene		sesquiterpene					*cruzi* - epima.	191.82	[53]
			infantum - proma. - intra. ama	117.54 261.25			*cruzi* - epima. - intra. ama	14.14 120.08	[36]
			infantum - proma.	5.19 ± 0.37					[9]
			major - proma.	6.51 ± 0.52					[9]
caryophyllene oxide		sesquiterpene					*brucei* - BSF	> 907.65	[55]

Compound	Type	Species / form	Value	Species / form	Value	Ref
		amazonensis - proma. - ama.	22.24 ± 2.3 19.97 ± 0.4			[18], [77]
(-)-*cis*-chrysanthenol	monoterpene			*cruzi* - epima.	> 665.69	[53]
1,8-cineole	monoterpene	*infantum* - proma. - intra. ama	> 648.30 > 648.30	*cruzi* - epima. - intra. ama.	4.08 > 648.30	[36]
		infantum - proma.	346.19 ± 0.98			[9]
		major - proma.	484.93 ± 1.66			[9]
		infantum - proma.	> 2593.19			[63]
		major - proma.	> 2593.19			[63]
		tropica - proma.	> 2593.19			[63]
citral	monoterpene			*brucei brucei* - BSF	39.28 ± 0.54	[38]
citronellal	monoterpene			*brucei brucei* - BSF	17.89 ± 1.55	[38]
citronellol	monoterpene	*infantum* - proma.	364.05 ± 1.45	*brucei brucei* - BSF	41.27 ± 4.86	[38], [9]
		major - proma.	398.92 ± 0.32			[9]
p-cymene	monoterpene	*infantum* - proma.	1163.62 ± 0.45	*brucei brucei* - BSF	568.66 ± 13.27	[38], [9]
		major - proma.	1633.04 ± 0.5			[9]
decanal	aldehyde	*donovani* - proma.	> 255.97			[50]
(*E*)-2-decenal	aldehyde	*donovani* - proma. - axe. ama - intra. ama	50.89 ± 0.28 16.01 ± 0.25 > 64.83			[50]
(*Z*)-4-decenal	aldehyde	*donovani* - proma.	> 259.32			[50]
(*Z*)-7-decenal	aldehyde	*donovani* - proma.	> 259.32			[50]
dodecanal	aldehyde	*donovani* - proma.	194.28 ± 3.11			[50]
(*E*)-2-dodecenal	aldehyde	*donovani* - proma. - axe. ama - intra. ama	23.86 ± 0.15 26.22 ± 1.12 52.66 ± 0.89			[50]
(*Z*)-8-undecenal	aldehyde	*donovani* - proma.	> 237.70			[50]
(*E*)-2-undecenal	aldehyde	*donovani* - proma. - axe. ama - intra. ama	16.70 ± 0.21 7.43 ± 0.11 33.57 ± 0.19			[50]
6,7-dehydroroyleanone	diterpene	*amazonensis* - proma.	7.79			[22]
eugenol	phenyl-propanoid	*infantum chagasi* - proma. - axe. ama - intra. ama	3045.07 1339.83 609.01			[79]
geraniol	monoterpene	*infantum* - proma.	24.51 ± 0.33			[9]
		major - proma.	36.11 ± 0.82			[9]
germacrone	sesquiterpene			*brucei brucei* - BSF	> 100	[10]

Compound	Class	Leishmania		Plasmodium		Trypanosoma		Ref
α-humulene	sesquiterpene	*donovani*	47.76					[60]
7-hydroxy-calamenene	sesquiterpene	*chagasi* - proma.	6.27					[61]
(*E*)-isoapiol	phenyl-propanoid			*falciparum* - multidrug resistant - chloroquine and antifolate sensitive	238.03 ± 2.9 314.52 ± 2.0			[52]
(*E*)-isoelemicin	phenyl-propanoid			*falciparum* - multidrug resistant - chloroquine and antifolate sensitive	> 96.04 > 96.04			[52]
isofuranodiene	sesquiterpene					*brucei brucei* - BSF	3.0 ± 0.8	[10]
(*E*)-isomyristicin	phenyl-propanoid			*falciparum* - multidrug resistant - chloroquine and antifolate sensitive	>520.26 >520.26			[52]
limonene	monoterpene	*infantum* - proma. - intra. ama	> 734.00 > 734.00			*cruzi* - epima. - intra. ama.	284.13 1071.20	[36]
						cruzi - epima. - trypoma.	247.36 ± 0.6 103.49 ± 2.1	[43]
						brucei - BSF	41.10 ± 1.6	[55]
						brucei brucei - BSF	31.12 ± 2.27	[38]
(-)-limonene epoxide	monoterpene					*brucei brucei* - BSF	19.3	[21]
(+)-limonene epoxide	monoterpene					*brucei brucei* - BSF	19.9	[21]
linalool	monoterpene	*infantum chagasi* - axe. ama. - intra. ama	3565.64 1348.46	*falciparum* - CQR	162 - 324	*cruzi* - trypoma.	2.01	[37], [79], [33]
		infantum - proma. - intra. ama	> 648.30 > 648.30			*cruzi* - epima. - intra. ama.	195.53 > 648.30	[36]
		braziliensis - proma.	2787.68					[69]
myrcene	monoterpene			*falciparum* - CQS - CQR	> 34.50 > 34.50	*brucei brucei* - BSF	16.44 ± 0.27	[38], [19]
nerol	monoterpene					*brucei brucei* - BSF	> 648.30	[38]
nerolidol	sesquiterpene	*braziliensis* - proma. - intra. ama.	334.13 213.61	*falciparum* - CQR	49.96			[37], [69]
(-)-perillyl alcohol	monoterpene					*brucei* - BSF	87	[21]
(-)-perillyl aldehyde	monoterpene					*brucei* - BSF	2.06	[21]
α-pinene	monoterpene	*infantum* - proma. - intra. ama	337.20 > 734.00	*falciparum* - CQS - CQR	> 34.50 > 34.50	*cruzi* - epima. - intra. ama.	20.11 14.09	[36], [19]
		infantum - proma.	129.18 ± 0.88					[9]
		major						[9]

Compound	Structure	Class	Leishmania sp. / form	Value	Trypanosoma sp. / form	Value	Ref.
			- proma.	145.33 ± 0.23			
			amazonensis				
			- proma.	144.60			[67]
			- axe. ama.	118.17			
			- intra. ama.	114.50			
β-pinene		monoterpene	*falciparum*		*brucei brucei*		[38],
			- CQS	> 34.50	- BSF	347.70 ± 15.65	[19]
			- CQR	> 34.50			
rotundifolone					*brucei*		[21]
					- BSF	1.93	
					cruzi		[43]
					- epima.	55.35 ± 1.8	
					- trypoma.	55.95 ± 1.5	
tetradecanal		aldehyde	*donovani*				[50]
			- proma.	150.67 ± 2.12			
thymol		monoterpene	*chagasi*				[73]
			- proma.	65.24			
thymoquinone		monoterpene	*tropica*				[41]
			- proma.	7.06 ± 0.05			
			- intra. ama	12.79 ± 0.05			
			infantum				[41]
			- proma.	8.95 ± 0.05			
			- intra. ama	15.83 ± 0.1			
(S)-*cis*-verbenol		monoterpene	*amazonensis*		*cruzi* - CL Brener strain		[76]
			- proma.	24.96	- trypoma.	54.52	
			braziliensis		*cruzi* -Ypsilon strain		[76]
			- proma.	13.79	- trypoma.	68.97	
			infantum				[76]
			- proma.	24.30			
zerumbone		sesquiterpene	*infantum*				[16]
			- proma.	41.22			

proma.: promastigote
ama.: amastigote
intra. ama.: intracellular amastigote
axe. ama.: axenic amastigote

CQS: chloroquine-sensitive
CQR: chloroquine-resistant

BSF: bloodstream form
epima.: epimastigote
trypoma.: trypomastigote

Table 2. *Essential oil components analyzed for in vitro anti-protozoal activities*

3. *In vivo* anti-protozoal activity

A lotion of the *Pistacia vera* L. EO at the concentration of 30 mg/mL reduced significantly (p < 0.05) the number of parasites in mice infected with *L. major* promastigotes compared to the control group after 30 days of daily rubbing treatment. Moreover, lesions size in treated mice decreased by 0.56 cm after 30 days of treatment, while an increase of 1.01 cm was observed in untreated group [46].

In BABL/c mice infected with *L. amazonensis*, five daily doses of the *Artemisia absinthium* L. EO at 30 mg/kg b.w. administered by intra-lesional route displayed significant control of lesion size and parasite burden (p < 0.05) compared to animals treated with a reference drug, glucantime and untreated mice [11].

In a model of experimental visceral leishmaniasis in BALB/c mice infected with *L. donovani* promastigotes, intra-peritoneal administration of 50, 100, 200 mg *Artemisia annua* L. EO/kg b.w. during 10 consecutive days lead to a 45.26 ± 8.95, 63.23 ± 7.28, 88.68 ± 5.52% decrease respectively of the parasitic load in the liver, while these reductions in the spleen were 72.48 ± 3.88, 80.72 ± 6.61, 91.66 ± 3.07% respectively [12].

In an animal model of cutaneous leishmaniasis, daily intra-peritoneal treatment of 30 mg *Bixa orellana* L. EO/kg/d during 14 days in BALB/c mice infected with *L. amazonensis* showed significant

reduction in the lesion size and the parasite load compared to mice treated with the vehicle or untreated [17].

The EO extracted from *Tetradenia riparia* (Hochst.) Codd leaves was analyzed for *in vivo* activity in mice infected with *L. amazonensis*. Daily topical application at concentrations of 0.85% and 1% for five weeks decreased the parasite load in the spleen compared to the negative control animals [24].

4. Mechanism of action

Different methods were used to approach the target and mode of action of EOs and their constituents.

4.1. *Direct effect on parasites*

- Changes in ultrastructure morphology of treated parasites were analyzed using scanning and transmission electron microscopy (SEM and TEM).

Treatment of *T. cruzi* trypomastigotes with EOs of *Annona vepretorum* Mart. (AV) and *Annona squamosa* L. (AS) for 24 hours at the IC_{50} (11.2 and 12.7 µg/mL respectively) and 2 times IC_{50} concentrations displayed significant alterations in the plasma membrane and mitochondrial swelling as observed by TEM. These effects were similar to known sterol biosynthesis inhibitors treatment [6].

Colares *et al.* determined morphological changes in *L. amazonensis* promastigotes treated with the *Vanillosmopsis arborea* Baker EO (a synonym of *Eremanthus arboreus* (Gardner) MacLeish) and its major component, α-bisabolol at 30 µg/mL (4 x IC_{50}) and 134.91 µM (6 x IC_{50}) respectively. After 24 hours of incubation, treated parasites exhibited cell damage, as evidenced by abnormal morphology, discontinuity of the nuclear membrane and lipid inclusion in the plasma membrane [15]. Using TEM to analyze alterations of *L. amazonensis* promastigotes treated with α-bisabolol at the concentration of 36.29 µM (IC_{50}), Rottini *et al.* determined different degrees of cell damage depending on the treatment length. After 2 hours, only mitochondrial swelling was observed, however after 4 hours, numerous vacuoles, lipid inclusions, condensed mitochondrial matrix and localization of the nucleus at the periphery were seen. After 16 hours, treated parasites showed nuclear membrane detachment, chromatin condensation, loss of cytoplasm organelles, lipid bilayer detachment of the plasma membrane and finally severe damage was observed after 24 hours [75].

Treatment with 250 µg/mL of the *Croton cajucara* Benth. EO (1 x MIC) caused cell damage on *L. chagasi* promastigotes with increased mitochondrial volume, loss of mitochondrial cristae, kinetoplast DNA fragmentation, condensation of nuclear chromatin and disorganization of the cytoplasmic organelles [61].

The EO of *Tetradenia riparia* (Hochst.) Codd at 0.03 µg/mL (IC_{50}) caused ultrastructural changes of *L. amazonensis* promastigotes: intense cytoplasm vacuolization, membranous profiles inside the organelles, lipid vesicles, membranes blebbing, thickening of the kinetoplast, chromatin condensation and nuclear fragmentation were observed [22].

L. infantum promastigotes treated with the EO of *Thymus capitellatus* Hoffmanns. & Link at the concentration of 37 µg/mL (IC_{50}) showed ultrastructure alterations. Using SEM, abnormal cell shape, irregular surface and impaired flagella were observed, while cytoplasmatic organelles disorganization, increased cytoplasmatic clearing and number of autophagosomal structures, swelling of cell body and mitochondria, nuclear chromatin disorganization, and cells with double nucleus were observed using TEM [63].

Severe morphological and ultrastructure changes were observed on *L. braziliensis* promastigotes treated with nerolidol, a major component of the *Piper aduncum* L. EO, at 334.13 µM (IC_{50}) and

668.26 µM (2 x IC$_{50}$) including shrinkage and roundness in the parasite cell body, mitochondrial swelling and disorganization, vesicles in the organelles and flagellar pocket. On the other side, little or no difference was noted after treatment with amphotericin B [69].

- Determination of cell apoptosis and necrosis using flow cytometry and fluorescence microscopy analyses

EOs extracted from *Artemisia annua* L. and *Syzygium aromaticum* (L.) Merr. & L.M.Perry demonstrated apoptosis induction on *L. donovani* promastigotes at 100 µg/mL (6.8 x IC$_{50}$ for *A. annua* and 6.6 x IC$_{50}$ for *S. aromaticum*). The externalization of phosphatidylserine (PS) (based on the annexin V/propidium iodide assay) was shown with 36.6 and 55.6% annexin V-positive promastigotes for *A. annua* and *S. aromaticum*, respectively, compared to 9.2 and 2.2% in untreated group. Moreover, EOs caused nuclear DNA fragmentation in treated parasites shown by the increase of fluorescence intensity, resulting from the binding between terminal deoxynucleotidyltransferase and fluorescence-labelled dUTP. The percentage of cells in the sub-G$_0$/G$_1$ phase also increased to 33.14 and 47.58% in parasites treated with *A. annua* and *S. aromaticum* EOs, respectively, compared to 1.69 and 1.20% in untreated group showing cell cycle arrest. Measurement of the loss of mitochondrial membrane potential was also used to determine cell apoptosis based on the decrease in JC-1-aggregates/monomers ratio. Treated *L. donovani* promastigotes showed a reduced ratio from 11.9 to 5.27 with *A. annua* EO and from 3.063 to 0.755 with *S. aromaticum* EO. These EOs also enhanced ROS levels in treated parasites which may be related to cell apoptosis [12], [32].

Using flow cytometry analysis, Aloui *et al.* showed effects of EOs extracted from *Artemisia herba-alba* Asso. (*A.ha*) and *Artemisia campestris* L. (*A.c*) on the cell cycle and cell apoptosis of *L. infantum* promastigotes. After 72 hours of incubation at concentrations of 68 - 460 µg/mL (IC$_{50}$ - 7 x IC$_{50}$) for *A.ha* and 44 - 220 µg/mL (IC$_{50}$ - 5 x IC$_{50}$) for *A.c*, the proportion of cells in the sub-G$_0$/G$_1$ phase increased to 33.2 - 95.5% (*A.ha*) and 27.0 - 96.5% (*A.c*) compared to 14.5 and 10.7%, respectively, of untreated group. Treatment at the same dose range in double annexin V/7-AAD staining assay caused an increase of 19.5 - 65.2% (*A.ha*) and 9.4 - 41.7% (*A.c*) in the percentage of annexin V-positive parasites, compared to < 2% in control groups. Parasites remained negative for 7-AAD in this assay [54].

Treatment of *T. cruzi* trypomastigotes with the EO of *Annona vepretorum* Mart. at 22.4 µg/mL (2 x IC$_{50}$) for 72 hours caused 20.5 and 44.9% of propidium iodide (PI)-positive and (PI + annexin V)-positive parasites, respectively, whereas only 1.18% of parasites were stained with annexin V indicating the necrosis effect of this EO on parasites [6].

Treatment at the concentration of 10 µg/mL of the *Chenopodium ambrosioides* L. EO (a synonym of *Dysphania ambroisioides* (L.) Mosyakin & Clemants) and its major compounds, ascaridole, carvacrol, caryophyllene oxide (2.7 x IC$_{50}$, 100 x IC$_{50}$, 0.7 x IC$_{50}$, and 2 x IC$_{50}$, respectively) indicated the absence of JC-1 aggregates in *L. amazonensis* promastigotes compared to parasites treated with the vehicle. This result suggested that mitochondrial dysfunction is involved in the anti-leishmanial effects of the EO as well as its major pure compounds [18].

The EO extracted from *Piper aduncum* L. did not change the number of *T. cruzi* epimastigotes at the concentration of 84.7 µg/mL (IC$_{50}$) in the G1 phase, however it reduced the number of cells in the G2 phase. This EO also decreased by approximately 98% the mitochondrial membrane potential [33].

The EO of *Thymus capitellatus* Hoffmanns. & Link at the concentration of 37 µg/mL (IC$_{50}$) increased the proportion of *L. infantum* promastigotes arrested in G$_0$/G$_1$ phase to 70% compared to 36% in untreated parasites. Percentage of parasites stained positively with annexin V was 16%, whereas this level was 3.3% in the untreated group. 20% of treated parasites had low mitochondrial membrane potential compared to 4% in the control group [63].

A sesquiterpene, α-bisabolol, demonstrated a disruption of 69.09% of the mitochondrial membrane potential at the concentration of 36.29 µM (IC$_{50}$) on *L. amazonensis* promastigotes compared to 17% in untreated cells [75].

Treatment of nerolidol at the concentration of 334.13 µM (IC$_{50}$) reduced mitochondrial membrane potential by 93% in the *L. braziliensis* promastigotes. Moreover, this compound caused the formation of a G$_0$/G$_1$ sub-peak of 30.36% compared to 0.53% in the negative control parasites. Flow cytometry analysis also showed that 82.2% of the parasites treated with nerolidol displayed positive labelling for both Annexin-V and PI in Annexin-V/PI double-labelled assay [69].

- Determination of enzyme inhibitory potential

The EO extracted from *Artemisia indica* Willd. demonstrated inhibitory potential against two *P. falciparum* type II fatty acid biosynthesis enzymes (*Pf*FabI and *Pf*FabZ) with IC$_{50}$ values of 32 and 41 µg/mL [13].

4.2. Immunomodulatory activity

- Determination of nitrite oxide (NO) production in macrophages

The EO of *Artemisia annua* L. leaves demonstrated a dose-dependent increase of NO production in macrophages infected with *L. donovani* promastigotes. At the highest concentration of 100 µg/mL (6.8 x IC$_{50}$), it caused the NO release of 8.96 ± 1.45 and 16.43 ± 1.68 µM in normal and infected macrophages compared to a lower level of NO in the control group [12].

Treatment of macrophages with the EO extracted from *Croton cajucara* Benth. at the concentration of 250 µg/mL (MIC against *L. chagasi* promastigotes) increased NO production by 41.1%. This EO at the same concentration increased NO production in macrophages infected with pre-treated parasites by 80.4%, whereas pre-infected macrophages with *L. chagasi* promastigotes followed by treatment with this EO showed an increase of 100% of NO level compared to the control group [61].

The EO of *Piper angustifolium* Lam. (a synonym of *Piper consanguineum* (Kunth) Steud) triggered the NO release of *L. infantum* infected macrophages after incubation at concentrations of 6.25 (4.35 x IC$_{50}$) and 12.5 µg/mL (8.7 x IC$_{50}$ obtained on *L. infantum* intracellular amastigotes). However this stimulation was not observed at higher concentrations (25 and 50 µg/mL) [34].

The EOs extracted from *Pistacia vera* L. and *Zataria multiflora* Boiss demonstrated the same type of effects on NO generation. Treated macrophages with these samples at the concentration of 3.125 µg/mL (0.15 x IC$_{50}$ for *P. vera* and IC$_{50}$ for *Z. multiflora* against *L. tropica* intracellular amastigotes) increased the NO release respectively to 16 µM and 18 µM compared to 11 µM in the untreated cells. However treatment with both EOs at higher concentrations (≥ 6.25 µg/mL) decreased the NO production to 6 µM [46], [65].

Both the *Syzygium cumini* (L.) Skeels EO and its major compound, α-pinene, exhibited a dose-dependent stimulation of NO production not only in normal but also in infected macrophages with *L. amazonensis* promastigotes after the treatment at concentrations ranging from 50 to 400 µg/mL [67].

- Quantification of cytokines

The EO extracted from *Tetradenia riparia* (Hochst.) Codd at the concentration of 0.03 µg/mL (IC$_{50}$ on amastigote form) changed cytokines production of macrophages infected with *L. amazonensis* promastigotes, as evidenced by the stimulation of interferon-γ and the inhibition of pro-inflammatory cytokines and cytokines induced by parasites infection, such as interleukin-1β (IL-1β), IL-17, IL-33, IL-10, IL-4, IL-5 and tumor necrosis factor [23].

- Determination of lysosomal and phagocytic activity of macrophages

The *Eugenia uniflora* L. EO at concentrations ranging from 3.12 to 12.5 µg/mL (IC_{50} to 4 x IC_{50} against *L. amazonensis* promastigotes) stimulated lysosomal activity of treated macrophages. However this activity decreased at higher concentrations (50 and 100 µg/mL) due to cytotoxicity (IC_{50} = 45.3 µg/mL against macrophages). Treatment of macrophages with this EO at concentrations ranging from 3.12 to 25 µg/mL also exhibited an increase of phagocytosis [29].

Rodrigues *et al.* also determined immunomodulatory effects of the EO extracted from *Syzygium cumini* (L.) Skeels and its major component, α-pinene. Treatment of macrophages with the EO at concentrations of 50, 100, 200 µg/mL (IC_{50} against *L. amazonensis* promastigotes was 60 µg/mL and IC_{50} against macrophages was 616.4 µg/mL) showed an increase of lysosomal activity, whereas it triggered phagocytosis at concentrations of 200 and 400 µg/mL. The enhancement of lysosomal effect and phagocytosis were also observed on macrophages treated with α-pinene at concentrations of 25, 50, 200 µg/mL and 100, 200, 400 µg/mL, respectively (IC_{50} against *L. amazonensis* promastigotes was 19.7 µg/mL and IC_{50} against macrophages was 425.2 µg/mL) [67].

5. Discussions and conclusion

157 EOs extracted from plants belonging to 28 families were reported for *in vitro* anti-parasitic activities against at least one parasite (*Leishmania, Plasmodium* or *Trypanosoma*) from 2013 until April 2017. Their number is classified according to plant families in Table 3. Lamiaceae and Piperaceae were the two most studied families with 30 and 27 tested EOs, respectively.

Family	n	Family	n	Family	n	Family	n
Lamiaceae	30	Rutaceae	6	Ranunculaceae	2	Euphorbiaceae	1
Piperaceae	27	Verbenaceae	5	Zingiberaceae	2	Hypericaceae	1
Asteraceae	17	Anacardiaceae	4	Bixaceae	1	Meliaceae	1
Myrtaceae	12	Lauraceae	4	Burseraceae	1	Plantaginaceae	1
Annonaceae	10	Fabaceae	3	Canellaceae	1	Scrophulariaceae	1
Apiaceae	10	Boraginaceae	2	Cannabaceae	1	Siparunaceae	1
Poaceae	9	Geraniaceae	2	Chenopodiaceae	1	Zygophyllaceae	1

Table 3. *Number of in vitro tested EOs against parasites (n) classified according to their families*

Studies mostly focused on anti-leishmanial effects, as evidenced by the 113 samples analyzed for this activity, followed by anti-trypanosomal effects with 56 tested EOs. The number of samples assayed for anti-plasmodial activity was only 22. Interestingly, only 17 EOs within the 157 samples in this paper were already mentioned in the previous review [4] and were analyzed against other parasite species or forms.

According to the classification defined before, 20 EOs extracted from 15 plants showed strong effect (IC_{50} < 2 µg/mL) against at least one parasite, while the number of samples revealing a moderate activity (2 µg/mL ≤ IC_{50} ≤ 20 µg/mL) was 58. The precise number of EOs exhibiting good, moderate or low activity against at least one species of *Leishmania* (a), *Plasmodium* (b) and *Trypanosoma* (c), classified according to plant families is shown in Figure 1.
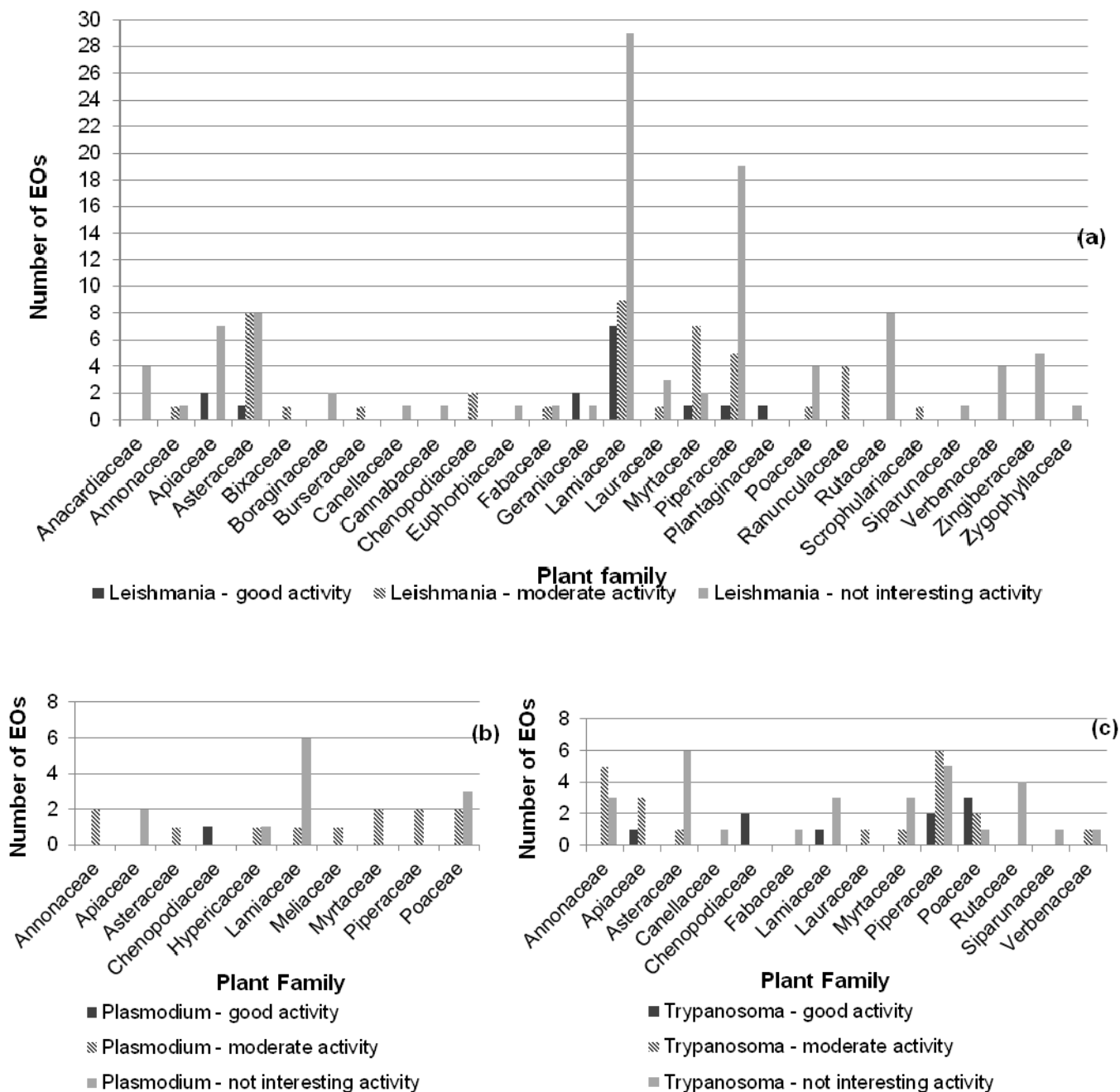
Figure 1. *Number of EOs showing good (IC$_{50}$ < 2 µg/mL), moderate (2 µg/mL ≤ IC$_{50}$ ≤ 20 µg/mL) or not interesting (IC$_{50}$ > 20 µg/mL) in vitro activity on at least one species of Leishmania (a), Plasmodium (b) and Trypanosoma (c), classified according to their plant families*

Similar to the previous review of Bero *et al.*, Lamiaceae is the most potential source of EOs with good and moderate activity against *Leishmania, Plasmodium* and *Trypanosoma*. Other interesting EOs belong mainly to Apiaceae, Asteraceae, Myrtaceae, Rutaceae and Poaceae families. We can also point out Piperaceae species showing notable parasiticidal effects not cited in the previous review.

The most active EOs (IC$_{50}$< 2µg/ml on at least one parasite and SI>10) are reported in Table 4. It is however difficult to compare these activities as models, testing methods and strains as well as mammalian cells used for SI often differ.

Family	Plant	IC$_{50}$ value (µg/mL)			SI (cell line) or IC$_{50}$ (µg/mL)	Ref.
		Leishmania (L.)	*Plasmodium (P.)*	*Trypanosoma (T.)*		
Apiaceae	*Ferula communis* L.	*L. infantum* proma.: 0.05 ± 0.01			81.60 (Raw 264.7)	[9]
		L. major proma.: 0.11 ± 0.04			37.09 (Raw 264.7)	
	Smyrnium olusatrum L.			*T. brucei* BSF: 1.97 ± 0.06	29 (BALB/c fibroblast)	[10]
Chenopodia-ceae	*Chenopodium ambrosioides* L.	*L. infantum*: 6.4 ± 0.6	*P. falciparum*: 0.2 ± 0.2	*T. brucei brucei*: 0.2 ± 0.07 *T. cruzi*: 1.9 ± 0.3	IC$_{50}$: 58.2 ± 0.05 (mouse peritoneal macrophage)	[18]
Geraniaceae	*Pelargonium graveolens* L'Hér.	*L. infantum* proma.: 0.11 ± 0.06			57.4 (Raw 264.7)	[9]
		L. major: proma.: 0.28 ± 0.08			22.5 (Raw 264.7)	
Lamiaceae	*Mentha crispa* L.			*T. brucei brucei* BSF: 0.33 ± 0.03	25 (HL-60)	[21]
	Teucrium polium L.	*L. infantum* proma.: 0.09 ± 0.02			40.44 (Raw 264.7)	[9]
		L. major proma.: 0.15 ± 0.09			24.26 (Raw 264.7)	
Myrtaceae	*Eugenia uniflora* L.	*L. amazonensis* proma.: 3.04 ± 0.75 *L. amazonensis* intra. ama.: 1.92 ± 0.8		*T. brucei brucei*: 11.20 ± 2.17	IC$_{50}$: 76.40 ± 11.95 (MCF-7) IC$_{50}$: 45.3 ± 2.45 (BALB/c macrophage)	[29], [30]
Piperaceae	*Piper angustifolium* Lam.	*L. infantum* intra. ama.: 1.43			33.72 (NIH/3T3) 22.15 (J774.A1)	[34]
	Piper brachypodon (Benth.) C. DC. - 2 samples			*T. cruzi* epima.: 0.34 and 1.74 *T. cruzi* intra. ama.: > 100 and 22.72	30.54 and 52.55 (Vero) 66.31 and 62.82 (THP-1)	[36]
Plantagina-ceae	*Octacanthus azureus* (Linden) Ronse	*L. amazonensis* axe. ama.: 0.7 *L. amazonensis* intra. ama.: 16.1			IC$_{50}$: 35.5 (BALB/c macrophage) IC$_{50}$: > 100 (Vero)	[14]
Poaceae	*Cymbopogon citratus* (DC.) Stapf. collected in Benin		*P. falciparum* CQS: 47.97 ± 13.09	*T. brucei brucei* BSF: 1.83 ± 0.13	IC$_{50}$: 10.63 ± 0.72 (CHO) IC$_{50}$: 39.77 ± 3.31 (WI38)	[38]
	Cymbopogon giganteus (Hochst.) Chiov.		*P. falciparum* CQS: 11.22 ± 5.35	*T. brucei brucei* BSF: 0.25 ± 0.11	IC$_{50}$ > 50 (CHO and WI38)	[38]
	Cymbopogon nardus (L.) Rendle			*T. brucei brucei*: 0.31 ± 0.03	> 323 (Vero)	[40]

proma.: promastigote
axe. ama.: axenic amastigote
intra. ama.: intracellular amastigote
BSF: bloodstream form

epima.: epimastigote
CQS: chloroquine-sensitive
CQR: chloroquine-resistant

Table 4. *The most active EOs against Leishmania, Plasmodium and Trypanosoma*

Although 157 EOs were reported for *in vitro* anti-parasitic activity and most of them tested for cytotoxicity on mammalian cells, only a few EOs were assayed for *in vivo* activity on animal models. It is the case for leishmanicidal effect of five EOs extracted from one Anacardiaceae species (*Pistacia vera* L.), two Asteraceae species (*Artemisia absinthium* L., *Artemisia annua* L.), one Bixaceae species (*Bixa orellana* L.) and one Lamiaceae species (*Tetradenia riparia* (Hochst.) Codd). Four of them were tested on cutaneous leishmaniasis model (*L. major* or *L. amazonensis*) while the *Artemisia annua* L. EO was tested on a visceral one (*L. donovani*).

Among these five EOs, two samples were studied to determine the target and/or mechanism of action. Treatment with the EO extracted from *Tetradenia riparia* (Hochst.) Codd not only affected ultrastructural morphology of *L. amazonensis* promastigotes but also changed cytokines production of infected macrophages. The *Artemisia annua* L. EO caused cell apoptosis on *L. donovani* promastigotes and its mode of action was supposed to be related to an increase in NO release of infected macrophages.

15 other EOs were analyzed for direct and/or immunomodulatory activity without any reports for *in vivo* activity, including two Annonaceae species (*Annona vepretorum* Mart., *Annona squamosa* L.), four Asteraceae species (*Artemisia campestris* L., *Artemisia herba-alba* Asso., *Artemisia indica* Willd., *Vanillosmopsis arborea* Baker), one Chenopodiaceae species (*Chenopodium ambrosioides* L.), one Euphorbiaceae species (*Croton cajucara* Benth.), two Lamiaceae species (*Thymus capitellatus* Hoffmanns & Link, *Zataria multiflora* Boiss), three Myrtaceae species (*Eugenia uniflora* L., *Syzygium aromaticum* (L.) Merr. & L.M.Perry, *Syzygium cumini* (L.) Skeels) and two Piperaceae species (*Piper aduncum* L., *Piper angustifolium* Lam. (a synonym of *Piper consanguineum* (Kunth) Steud)).

Chemical composition of tested EOs are also important to analyze, as it is well known that EOs are complex mixtures of several volatile compounds and their constituents vary according to many factors, such as plant environment and growing conditions, methods of harvesting, extraction and storage. Moreover, the major component of an EO can also vary in different chemotypes of the same plant species. This chemical variability can influence their activities or adverse effects. Therefore, a clear knowledge of the EO composition is necessary [3]. Interestingly, most of effective EOs described in the section 2.1 were analyzed by gas chromatography to identify their constituents. Only six EOs whose activity is described in Houël *et al.* [14] were not analyzed because of their less interesting effect compared to other samples. The analytical methods as well as the three major components of each sample are listed in Table 5.

Family	Plant	Chemical analysis		Ref.	Plant	Chemical analysis		Ref.
		Method	Major compounds* (%)			Method	Major compounds* (%)	
Annonaceae	*Annona vepretorum* Mart.	GC-MS GC-FID	bicyclogermacrene (39.0) spathulenol (14.0) α-phellandrene (11.5)	[6]	*Xylopia frutescens* Aubl.	GC-MS GC-FID	(*E*)-caryophyllene (24.8) bicyclogermacrene (20.8) germacrene D (17.0)	[8]
	Annona squamosa L.	GC-MS GC-FID	(*E*)-caryophyllene (27.4) germacrene D (17.1) bicyclogermacrene (10.8)	[6]	*Xylopia laevigata* (Mart.) R. E. Fries (collected in Mata do Crasto)	GC-MS GC-FID	germacrene D (18.9) bicyclogermacrene (18.4) β-elemene (9.5)	[8]
	Bocageopsis multiflora (Mart.) R. E. Fries	GC-MS GC-FID	β-bisabolene (13.2) spathulenol (13.0) caryophyllene oxide (12.6)	[7]	*Xylopia laevigata* (Mart.) R. E. Fries (collected in Serra de Itabaiana)	GC-MS GC-FID	germacrene D (27.0) bicyclogermacrene (12.8) (*E*)-caryophyllene (8.6) γ-muurolene (8.6)	[8]
Apiaceae	*Ferula communis* L.	GC-MS GC-FID	**(*E*)-caryophyllene (15.22)** myrcene (10.33) α-eudesmol (9.8)	[9]	*Smyrnium olusatrum* L. - leaves	GC-MS	furanoeremophil-1-one (30.0) **germacrone (9.7)** β-pinene (9.5)	[10]
	Smyrnium olusatrum L. - fruits	GC-MS	**1β-acetoxy-furanoeudesm-4(15)-ene (31.2) isofuranodiene (6.6)** β-phellandrene (6.2)	[10]	*Smyrnium olusatrum* L. - roots	GC-MS	furanoeremophil-1-one (24.4) β-phellandrene (14.4) **isofuranodiene (5.8)**	[10]
	Smyrnium olusatrum L. - flowers	GC-MS	myrcene (18.2) furanoeremophil-1-one (12.1) **germacrone (10.4)**	[10]				
Asteraceae	*Artemisia campestris* L.	GC-MS GC-FID	**α-pinene (24.98)** β-pinene (24.74) myrcene (7.78)	[9]	*Artemisia indica* Willd.	GC-MS GC-FID	camphor (13.0) α-thujone (7.37) borneol (6.97)	[13]
	Artemisia absinthium L.	GC-MS	*trans*-sabinyl acetate (36.7) *p*-cymen-7-ol (5.4) *trans*-sabinol (5.0)	[11]	*Vanillosmopsis arborea* (Gardner) Baker	GC-MS	**α-bisabolol (97.9)** *o*-methyl eugenol (1.6) bisabolol oxide (0.5)	[15]
	Artemisia annua L.	GC-MS	camphor (52.06) (*E*)-caryophyllene (10.95) 1,8-cineole (5.57)	[12]	*Vernonia polyanthes* Less.	GC-MS	myrcene (34.3) **zerumbone (15.8)** bicyclogermacrene (8.9)	[16]
	Artemisia herba-alba Asso	GC-MS GC-FID	**camphor (31.51)** fenchol (13.85) α-thujene (11.62)	[9]				

Family	Species	Method	Components	Ref.	Species	Method	Components	Ref.
Bixaceae	*Bixa orellana* L.	GC-MS	ishwarane (18.6) geranylgeraniol (9.1) bicyclogermacrene (8.4)	[17]				
Chenopodia-ceae	*Chenopodium ambrosioides* (L.)	GC-MS GC-FID	**carvacrol (62.36) ascaridole (22.54) caryophyllene oxide (5.64)**	[18]				
Geraniaceae	*Pelargonium graveolens* L'Hér.	GC-MS GC-FID	**citronellol (24.75) geraniol (13.99)** γ-eudesmol (11.23)	[9]				
Hyperica-ceae	*Hypericum scabrum* L.	GC-MS	**α-pinene (74.0) β-pinene (4.8) myrcene (3.4)**	[19]				
Lamiaceae	*Teucrium polium* L. (collected in Saudi Arabia)	GC-MS	(E)-3-caren-2-ol (12.1) terpinen-4-ol (5.8) (E)-pinocarveol (5.4)	[20]	*Tetradenia riparia* (Hochst.) Codd - spring	GC-MS GC-FID	14-hydroxy-9-*epi*-caryophyllene (24.36) calyculone (15.64) *cis*-muurolol-5-en-4-α-ol (13.2)	[22]
	Teucrium polium L. (collected in Northern Tunisia)	GC-MS GC-FID	**carvacrol (56.06) (E)-caryophyllene (7.68) 1,8-cineol (6.26)**	[9]	*Tetradenia riparia* (Hochst.) Codd - summer	GC-MS GC-FID	14-hydroxy-9-*epi*-caryophyllene (18.27) fenchone (12.67) *cis*-muurolol-5-en-4-α-ol (11.74)	[22]
	Mentha australis R. Br.	GC-MS	β-linalool (22.9) 3,7-octadien-2,6-diol,2,6-dimethyl- (8.6) *t*-gurjenene (8.1)	[20]	*Tetradenia riparia* (Hochst.) Codd - fall	GC-MS GC-FID	14-hydroxy-9-*epi*-caryophyllene (20.34) *cis*-muurolol-5-en-4-α-ol (13.78) calyculone (12.58)	[22]
	Mentha microphylla K. Koch.	GC-MS	carvone (64.6) limonene (19.5) cineol (4.3)	[20]	*Tetradenia riparia* (Hochst.) Codd - winter	GC-MS GC-FID	calyculone (24.7) abietadiene (13.54) 14-hydroxy-α-muurolene (7.44)	[22]
	Rosmarinus officinalis L.	GC-MS GC-FID	**1,8-cineol (43.77) camphor (11.96) α-pinene (11.52)**	[9]	*Tetradenia riparia* (Hochst.) Codd - spring	GC-MS GC-FID	α-cadinol (13.81) 14-hydroxy-9-*epi*-caryophyllene (12.70) 6,7-dehydroroyleanone (12.51)	[24]
	Salvia officinalis L.	GC-MS GC-FID	**camphor (25.13)** α-thujene (21.47) **1,8-cineol (16.43)**	[9]	*Tetradenia riparia* (Hochst.) Codd - summer	GC-MS GC-FID	α-cadinol (16.91) 14-hydroxy-9-*epi*-caryophyllene (15.28) 6,7-dehydroroyleanone (14.00)	[24]
	Thymus hirtus Banks & Sol.	GC-MS GC-FID	**α-pinene (16.93) 1,8-cineol (16.13)** β-pinene (8.78)	[9]	*Tetradenia riparia* (Hochst.) Codd - fall	GC-MS GC-FID	α-cadinol (17.16) 6,7-dehydroroyleanone (16.50) 14-hydroxy-9-*epi*-caryophyllene (13.10)	[24]
	Mentha crispa L.	GC-MS	**rotundifolone (58.11)** limonene (10.58) myrcene (7.79)	[21]	*Tetradenia riparia* (Hochst.) Codd - winter	GC-MS GC-FID	6,7-dehydroroyleanone (20.47) α-cadinol (14.82) 14-hydroxy-9-*epi*-caryophyllene (10.23)	[24]
Lauraceae	*Cinnamomum verum* J.Presl.	GC-MS	(E)-cinnamaldehyde (81.52) eugenol (16.68) (E)-caryophyllene (1.19)	[25]	*Laurus nobilis* L.	GC-MS GC-FID	**1,8-cineol (43.35)** sabinene (9.39) fenchol (6.75)	[9]
Meliaceae	*Cedrelopsis grevei* Baill. & Courchet	GC-MS GC-FID	(E)-β-farnesene (27.67) δ-cadinene (14.52) α-copaene (7.67)	[26]				
Myrtaceae	*Eucalyptus*	GC-MS	**1,8-cineol (42.31)**	[9]	*Eugenia uniflora*	GC-MS	curzerene (47.3)	[29]

	globulus Labill.	GC-FID	limonene (12.91) *α*-terpineol (6.74)		*L*. collected in Brazil	GC-FID	*γ*-elemene (14.25) *trans-β*-elemenone (10.4)	
	Myrtus communis L.	GC-MS GC-FID	**α-pinene (52.52) 1,8-cineol (24.32)** limonene (6.89)	[9]	*Eugenia uniflora* L. collected in Egypt	GLC-MS GLC-FID	spathulenol (15.8) *α*-copaene (10.96) muurola-4,10-dien-1*β*-ol (9.3)	[30]
	Myrtus communis L.	GC-MS GC-FID	*α*-pinene (24.7) 1,8-cineol (19.6) linalool (12.6)	[27]	*Plinia cerrocampanensis* Barrie	GC-MS	*β*-bisabolene (16.26) linalool (10.47) *α*-copaene (8.86)	[31]
	Eugenia pitanga (O.Berg) Nied.	GC-MS GC-FID	spathulenol (15.34) globulol (10.93) (2*E*,6*E*)-methyl farnesoate (7.40)	[28]	*Syzygium aromaticum* (L.) Merr. & L. M. Perry	GC-MS	eugenol (59.75) eugenyl acetate (29.24) (*E*)-caryophyllene (3.75)	[32]
Piperaceae	*Piper aduncum* L.	GC-MS	nerolidol (25.22) **linalool (13.42)** spathulenol (6.29)	[33]	*Piper bogotense* C. DC.	GC-MS GC-FID	*trans*-hydrate sesquisabinene (14.2) *α*-phellandrene (13.7) **α-pinene (8.7)**	[36]
	Piper angustifolium Lam.	GC-MS	spathulenol (23.78) caryophyllene oxide (13.06) *α*-pinene (5.87)	[34]	*Piper marginatum* Jacq.	GC-MS GC-FID	oxygenated sesquiterpene I (18.4) *α*-phellandrene (11.2) ***trans-β-caryophyllene (11.1)***	[36]
	Piper arboreum Aubl.	GC-MS	(*E*)-caryophyllene (12.6) 1-*epi*-cubenol (10.4) *trans*-cadina-1(6),4-diene (9.6)	[35]	*Piper divaricatum* G. Mey.	GC-MS GC-FID	**1,8-cineol (18.3) linalool (15.0) α-pinene (11.4)**	[36]
	Piper diospyrifolium Kunth	GC-MS	selin-11-en-4-*α*-ol (17.7) (*E*)-caryophyllene (7.4) *γ*-gurjunene (6.9)	[35]	*Piper septuplinervium* (Miq.) C. DC.	GC-MS GC-FID	*δ*-cadinene (10.9) *epi*-cubebol (9.0) viridiflorol (7.9)	[36]
	Piper mosenii C. DC.	GC-MS	caryophyllene oxide (12.1) *α*-humulene (11.3) (*E*)-caryophyllene (8.6)	[35]	*Piper lanceifolium* Kunth	GC-MS GC-FID	***trans-β-caryophyllene (11.6)*** germacrene D (10.7) *β*-selinene (7.8)	[36]
	Piper rivinoides Kunth	GC-MS	bicyclogermacrene (11.8) (*Z*)-*α*-bisabolene (10.9) *α*-humulene (10.0)	[35]	*Piper claussenianum* (Miq.) C. DC.	GC-MS GC-FID	**linalool (56.5) nerolidol (23.7)** *α*-humulene (2.4)	[37]
	Piper brachypodon (Benth.) C. DC. (collected in Tutunendo)	GC-MS GC-FID	***trans-β-caryophyllene (20.2)*** caryophyllene oxide (10.8) bicyclogermacrene (8.1)	[36]	*Piper lucaeanum* var. *grandifolium* Yunk	GC-MS GC-FID	*α*-zingiberene (30.4) *α*-pinene (30.0) *β*-sesquiphellandrene (11.1)	[37]
	Piper brachypodon (Benth.) C. DC. (collected in Salero)	GC-MS GC-FID	***trans-β-caryophyllene (20.2)*** caryophyllene oxide (10.7) bicyclogermacrene (8.5)	[36]	*Piper hispidum* Sw.	GC-MS	curzerene (15.7) germacrene B (10.9) *α*-selinene (10.5)	[14]
Poaceae	*Cymbopogon citratus* (DC.) Stapf collected in Benin	GC-MS GC-FID	**geranial (39.5) neral (35.5) β-pinene (10.1)**	[38]	*Cymbopogon schoenanthus* (L.) Spreng.	GC-MS GC-FID	piperitone (60.3) (+)-2-carene (13.0) **limonene (6.4)**	[38]
	Cymbopogon citratus (DC.) Stapf collected in Cameroon	GC-MS GC-FID	geranial (32.82) neral (30.21) myrcene (11.43)	[39]	*Cymbopogon nardus* (L.) Rendle collected in Benin	GC-MS GC-FID	**β-citronellal (35.9) nerol (24.3) β-citronellol (11.6)**	[38]
	Cymbopogon giganteus Chiov.	GC-MS GC-FID	*trans-p*-mentha-1(7),8-dien-2-ol (18.3) *trans*-carveol (17.4) *cis-p*-mentha-2,8-dienol (11.3)	[38]	*Cymbopogon nardus* (L.) Rendle collected in Malaysia	GC-MS GC-FID	citronellal (35.5) geraniol (28) citronellol (11)	[40]
Ranunculaceae	*Nigella sativa* L. collected in	GC-MS GC-FID	**p-cymene (53.10)** *o*-cymene (18.50)	[9]	*Nigella sativa* L. collected in Iran	GC-MS GC-FID	**thymoquinone (42.4)**	[41]

			Tunisia		α-thujene (7.20)			p-cymene (15.1) carvacrol (12.3)
Rutaceae	*Citrus aurantium* L.	GC-MS GC-FID	limonene (94.4) β-pinene (1.9) β-myrcene (1.1)	[42]	*Swinglea glutinosa* (Blanco) Merr.	GC-MS GC-FID	germacrene D (30.8) β-pinene (22.6) sabinene (11.6)	[42]
Verbenaceae	*Lippia pedunculosa* Hayek	GC-MS GC-FID	**rotundifolone (71.7)** **limonene (21.8)** piperitenone (1.2)	[43]				

*: compounds in bold are those tested for anti-parasitic activity in the same study

Table 5. *Analytical methods and major components of interesting EOs*

Regarding anti-parasitic activities of essential oil components, 41 pure compounds isolated from EOs or purchased from commercial suppliers were evaluated for the first time and 10 for a second time [4]. Among them, 3 compounds (carvacrol against *L. chagasi*, citronellal and limonene epoxide against *T. brucei brucei*) showed a better effect, while 6 (α-humulene against *L. donovani*, limonene against *T. cruzi*, linalool against *P. falciparum*, α-pinene against *L. major*, β-pinene against *P. falciparum* and *T. brucei brucei*, thymol against *L. chagasi*) were in the same range and 2 (nerolidol and α-pinene against *P. falciparum*) showed a lower effect.

It is difficult to explain the activity of the most active EOs by a specific component because most identified compouds were not tested and tested ones are often less active than the corresponding EO, suggesting synergistic or additive effects. Up to now, conclusions can only be drawn for two compounds: ascaridole, the major component of *Chenopodium ambrosioides* L., and rotundifolone, the one of *Mentha crispa* L., showing a strong effect against *L. amazonensis* and *T. brucei,* respectively. Indeed, these identified compounds can explain an important part of EOs activity. 19 other pure compounds showed moderate activities against at least one parasite. Among them, thymoquinone, the major compound of *Nigella sativa* L. EO, was active against both *L. tropica* and *L. infantum,* and β-caryophyllene, a very popular sesquiterpene, was reported for *in vitro* activity against three parasite species, *L. tropica, L. infantum* and *T. cruzi*. All these compounds were examined for cytotoxicity and five of them, namely ascaridole, α-bisabolol, caryophyllene oxide, 6,7-dehydroroyleanone and linalool, showed toxicity on mammalian cells, but no detailed safety or benefit/risk analyses were realized.

Furthermore, none of the 21 compounds with interesting *in vitro* effect was reported for *in vivo* activity alone. In one research, to increase anti-leishmanial effect and reduce toxicity, three compounds, ascaridole, carvacrol and caryophyllene oxide, were combined and tested for *in vitro* and *in vivo* activity against *L. amazonensis*. The combination of ascaridole - carvacrol at the ratio 1: 4 revealed the highest synergistic activity and the lowest cytotoxicity *in vitro*. Treatment of infected BALB/c mice with this combination at the dose of 20:80 mg/kg presented lower (p < 0.05) lesion size and parasite burden compared to the control and vehicle treated groups [77]. Interestingly, similar to the *Chenopodium ambrosioides* L. EO (a synonym of *Dysphania ambrosioides* (L.) Mosyakin & Clemants), the anti-leishmanial activity of its major compounds, ascaridole, carvacrol and caryophyllene oxide, was explained by a breakdown of mitochondrial membrane potential of treated parasites.

Concerning the mode of action of pure compounds against parasites, plasma and nuclear membranes were identified as the targets of α-bisabolol. This compound also caused cell apoptosis in *L. amazonensis*. Another sesquiterpene, nerolidol, affected the mitochondrial membrane of *L. braziliensis* as evidenced by structural alterations and potential variations observed by TEM, SEM and in flow cytometry assay respectively. In the case of α-pinene, the activity against *L. amazonensis* was proposed to be related to an increase of NO production of infected macrophages but also the enhancement of lysosomal effect and phagocytosis of treated macrophages.

This review shows clearly the potential of EOs and their components as effective alternatives to fight against *Leishmania, Plasmodium* and *Trypanosoma*. However, more studies are needed especially

in vivo assessments, toxicity evaluation and mechanism of action determination , so that they can be used as effective treatments.

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