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Review

Antiprotozoa activity of some essential oils

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Protozoa cause many diseases such as leishmaniasis, giardiasis, amebiasis, trichomoniasis and Chagas disease. Metronidazole is the most commonly used drug in antiprotozoal therapy, but metronidazole, as well as other antiprotozoal drugs, can have several secondary effects. Essential oils have been successfully used in the treatment of diseases caused by protozoa. This is a review covering such research from the last 10 years. The main essential oils from 42 plant species demonstrate activity against protozoa, and those from the Lamiaceae family were the most studied with 16 species showing antiprotozoal effects. Other plant families containing species that demonstrate therapeutic effects include Verbenaceae with seven species, Asteraceae with four, Piperaceae with three and Annonaceae with two; the others nine families each had one species. Thymol was the main component found in eight species, followed by eugenol and terpinen-4-ol, which were found in four species, and carvacrol and camphor, which were identified in three and two species, respectively.

Key words: Essential oil, diseases, Protozoa.

INTRODUCTION

Protozoa are single-celled organisms found as ubiquitous, free-living organisms in the environment. As eukaryotes, protozoa have a membrane-bound nucleus with well-defined chromosomes. Protozoal diseases, such as amebiasis, giardiasis, trichomoniasis, leishmaniasis and trypanosomiasis, constitute a major public health problem in Latin American countries.

Protozoa are classified as sporozoa (e.g., *Plasmodium* species) which are intracellular parasites; flagellates (e.g., *Giardia lamblia, Leishmania infantum, Trichomonas vaginalis* and *Trypanosoma cruzi*) that possess tail-like structures for movement; amoeba (e.g., *Entamoeba histolytica*) that move using temporary cell body projections termed pseudopods or ciliates, which move by beating multiple hair-like structures called cilia. Infections caused by protozoa can occur through ingestion of cysts, sexual transmission, or through insect vectors.

The highest prevalence of *E. histolytica* is in developing countries. Although amebiasis can be asymptomatic, this parasite generally causes dysentery, and invasive extraintestinal diseases can occur, resulting in abscesses in the liver and, in some cases, the involvement of other organs. Amebiasis ranks third worldwide in lethal infection (Walsh, 1988; Petri and Mann, 1993).

G. lamblia has two morphologic forms: Cysts and trophozoites (Bernard et al., 2001). Cysts, in particular, are responsible for fecal-oral transmission, as they are viable in cold water for several months (Bingham et al., 1979). This protozoon is responsible for intestinal infection and diarrhea that may lead to nutritional deficiencies, especially in children. Its disease incidence was reported to be 4.6 million episodes, with 2.2 million deaths due to diarrhea per year; of these, 1.8 million deaths were in developing countries (World Health Organization (WHO), 2004). The abundance of *Giardia* in people, animals, and environmental sources suggests that eradication is not an option.

Trichomoniasis is caused by *T. vaginalis* and is the most common non-viral, asexually transmitted infection, with approximately 170 million newly acquired infections worldwide per year. It has been linked to an increase in the risk of HIV transmission (Petrin et al., 1998) as well as adverse pregnancy outcome, low birth weight, infertility, postoperative infections and cervical neoplasia

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(Patel et al., 2000; Schwebke and Burgess, 2004). Some men who are infected with *T. vaginalis* may not display symptoms but are still infectious carriers of this parasite.

The most effective and commonly used drug in the treatment of these three protozoans is metronidazole. However, this compound has unpleasant side effects such as a metallic taste, headache, dry mouth, urticaria, pruritus, and dark-colored urine. In addition, carcinogenic, and teratogenic effects have been documented (Upcroft et al., 1999; Upcroft and Upcroft, 2001). These potential toxic and mutagenic effects are the main reasons why it is essential to develop alternative antiprotozoal agents with high activity, low toxicity, and high efficacy.

The *Leishmania* life cycle begins when an infected fly bites and injects the protozoa promastigotes into the skin of the new host. These promastigotes are then phagocytosed by macrophages and transform into amastigotes, which are able to divide. When the macrophages burst, they release more amastigotes that are again phagocytosed to continue the process of infection.

Leishmaniasis includes two major diseases: Cutaneous leishmaniasis and visceral leishmaniasis. Cutaneous leishmaniasis, the most common form of the disease, produces skin ulcers and is caused by more than 20 *Leishmania* species. Visceral leishmaniasis causes a severe systemic disease that is typically fatal when left untreated. This type of leishmaniasis is caused by *Leishmania chagasi/infantum*, which is an emerging opportunistic disease in patients with AIDS (Desjeux, 2004).

The treatment of leishmaniasis depends on the form of the disease. Cutaneous leishmaniasis sometimes heals on its own and may not require treatment, while visceral leishmaniasis is treated with pentavalent antimonial drugs or amphotericin B that may produce side effects, such as weakness, headache, dizziness, cardiotoxicity, etc.

Another parasitosis of major importance on the American continent is caused by *T. cruzi*. Chagas disease is named after the Brazilian physician Carlos Chagas, who discovered the disease in 1909. It is caused by the *T. cruzi* protozoa, which is transmitted to animals and people through insect vectors, and is also referred to as American trypanosomiasis.

The *T. cruzi* life cycle starts in an animal reservoir, usually wild or domestic mammals, including humans. A reduviid bug serves as vector. Inside of this vector, the protozoa enter the epimastigote stage. After reproduction, the epimastigotes move onto the rectal cell wall, where they then transform into infectious tripomastigotes. Then, when the reduviid bug drinks blood from a human, it defecates, releasing the tripomastigotes contained in its feces and allowing the protozoa to enter the human host through the bite wound or by crossing mucus membranes. The molecules and proteins on the cytoskeleton of the host cell bind to the surface of the parasite and initiate invasion (Ley et al., 1988). When they enter a human cell, the *T. cruzi* become amastigotes and reproduce within the cell. Chagas disease also can be acquired by humans through blood transfusions and organ transplantation (Nda et al., 2010), congenitally (that is, from a pregnant woman to her baby) (Gürtler et al., 2003), and through oral contamination (e.g., foodborne parasite transfer) (Nóbrega et al., 2009). Acute infection can be lethal, and cardiomyopathy develops in 25 to 30% of infected persons (PAHO, 2010). The treatment of this disease has, until recently, utilized nifurtimox and benznidazole (Rassi and Luquetti, 1992). Treatment of *T. cruzi* is not very effective, and the drugs often produce side effects.

Plants and their extracts and essential oils have been used for many years in the treatment of several diseases, including parasite infections (Jones, 1996). The essential oils can be extracted from different parts of the plant, obtained by crushing or by distillation.

We performed a literature review of the essential oils that have been shown to possess activity against *E. histolytica, G. lamblia*, *T. vaginalis*, *T. cruzi*, and/or *Leishmania* sp.

ESSENTIAL OILS AGAINST *ENTAMOEBA HISTOLYTICA*

Thymus vulgaris L. (Lamiaceae)

The antiamoebic effects of hydroalcoholic and n-hexane extracts, as well as of the essential oil, of T. vulgaris against *E. histolytica* have been tested. The minimal inhibitory concentration (MIC) of this plant's hydroalcoholic extract, hexane extract, and essential oil after 24 h was 4.0, 4.0, and 0.7 mg/ml, respectively (Behnia et al., 2008a). The main components of the essential oil are thymol, carvacrol, borneol, and linalool (Goodner et al., 2006), and the antiamoebic activity of the plant appears to be attributable to these compounds.

Allium sativum L. (Amaryllidaceae)

The antiamoebic properties of the hydroalcoholic and hexanic extracts and the essential oil of Iranian *A. sativum* (garlic) were studied, with MICs after 24 h of 60.0, 4.0, and 0.4 mg/ml, respectively. After 48 h, the MICs for the hexane extract and essential oil were 3 and 0.3 mg/ml, respectively, whereas metronidazole had a MIC of 2 µg/ml after 24 h and 1.5 µg/ml after 48 h. These results indicate that longer periods of exposure to the essential oil decreased the number of viable trophozoites (Behnia et al., 2008b).

ESSENTIAL OILS AGAINST *G. LAMBLIA*

The antigiardial activity of the phenol-rich essential oils of

Thymbra capitata L. (Lamiaceae), *Origanum virens* Hoffmanns (Lamiaceae), *Thymus zygis* subsp. Sylvestris chemotype Thymol Hoffmanns and Link (Lamiaceae), and *Lippia graveolens* Kunth (Verbenaceae) were analyzed. Their activity was evaluated based on the change in parasite growth, cell viability, adherence, and morphology. All of the essential oils tested inhibited the growth of *G. lamblia*, and the *T. capitata* oil was the most active. The oil, at IC_{50} values in the range 71 to 257 µg/ml, inhibited parasite adherence since the first hour of incubation and, killed almost 50% of the parasites. The ultrastructural alteration produced by the essential oils were deformations in trophozoite appearance, the presence of membrane blends, electrodense precipitates in the cytoplasm and nuclei, and the internalization of flagella and ventral discs. None of these essential oils produced cytotoxic effects in mammalian cells, suggesting that the oils of *T. capitata, O. virens, T. zygis* Sylvestris chemotype Thymol, and *L. graveolens* have great potential as treatment agents for diseases caused by *G. lamblia* (Machado et al., 2010a).

In another study, eighteen essential oils were tested against *G. lamblia*, and the most active oils were those containing phenols. In particular, essential oils from species such as *T. capitata* and *O. virens*, which contain a high content of carvacrol, showed greater activity, with IC_{50} values of 71 and 85 µg/ml, respectively. These values are in agreement with the results obtained in the study aforementioned. The oils from *Syzygium aromaticum* L. Merr and L. M. Perry (Myrtaceae) and *T. zygis* subsp. sylvestris (Hoffmanns and Links) Cout. (Lamiaceae) (IC_{50} from 100 to 200 µg/ml) and the oils from *Mentha* × *piperita* (Lamiaceae) and *L. graveolens* (IC_{50} over 200 µg/ml) were less active. From the eighteen essential oils tested, more than one hundred different compounds were identified. Forty-seven compounds were found in concentrations over 2.0%. The main components were α-pinene, myrcene, limonene, *p*-cymene, 1, 8-cineole, camphor, and borneol (Machado et al., 2010b). In another study, the antigiardial activity of *S. aromaticum* essential oil was evaluated, and its chemical composition shows that eugenol was the major component (Santoro et al., 2007). The species antigiardial effect on parasite growth, adherence, viability, and ultrastructure were studied.

The essential oil (IC_{50} 134 µg/ml) and eugenol (IC_{50} 101 µg/ml) were found to have antiprotozoal effects. The trophozoites adherence was reduced within the first hour of incubation, and eugenol inhibited trophozoite adherence starting from the third hour, without inducing cell lysis. The main morphological alterations were modifications in the cell shape, the presence of precipitates in the cytoplasm, autophagic vesicles, internalization of flagella and ventral discs, membrane blends, and intracellular and nuclear clearing. These findings indicate that the essential oil and eugenol have antigiardial activity (Machado et al., 2011).

Ocimum basilicum L. (Lamiaceae)

The effects of *O. basilicum* essential oil on *G. lamblia* and on the interaction of these parasites with peritoneal mouse macrophages were investigated. The composition of the essential oil was also determined. The main components of the oil were linalool (69.33%) and eugenol (10.85%). The essential oil (2 mg/ml) killed nearly 80% of the parasites within 120 min; at this concentration, the mouse macrophages showed little decrease in viability. At 300 µg/ml of linalool and 850 µg/ml of eugenol, the purified fractions killed 100 and 70% of the parasites, respectively, in 60 min, with no damage to macrophages. Pretreatment of peritoneal mouse macrophages with 2 mg/ml essential oil reduced 79% of the association index between the macrophages and *G. lamblia*, with a concomitant increase of 153% in nitric oxide production by the *G. lamblia* (Almeida et al., 2007).

The effect of the three phenolic compounds, eugenol, thymol, and carvacrol, which are commonly found in many essential oils, on *G. lamblia* growth and adherence were tested. The concentrations that inhibit 50% of parasite growth (IC_{50}) were determined by total cell counting. For the study of *Giardia* adherence, the trophozoites were exposed to pure phenolic compounds at IC_{50} concentrations for 7 h at 37°C. The number of unattached and attached cells was determined microscopically. The growth of the parasite was significantly inhibited by the three compounds, with the most active being thymol (IC_{50} 47 µg/ml), followed by carvacrol (IC_{50} 50 µg/ml) and eugenol (IC_{50} 131 µg/ml). The three phenolic compounds inhibited *G. lamblia* trophozoite adherence from the first hour of incubation. These results suggest that carvacrol, thymol, and eugenol have antigiardial activity (Machado et al., 2008).

Carum copticum (L.) Benth and Hook f. (Apiaceae)

For many years, *C. copticum* has been used as an antibacterial, antifungal, and antiprotozoal. The alcoholic extract and the essential oil of this plant were tested against *G. lamblia* cysts. After 60 min of incubation, the MICs of the extract and the oil were 100 and 8 mg/ml, respectively. After 120 min, the MICs were 75 and 6 mg/ml, and after 180 min, the MICs of the alcoholic extract and the essential oil were 75 and 4 mg/ml, respectively. These results indicate that *C. copticum* is effective against *G. lamblia in vitro* (Shahabi et al., 2008).

Lavandula angustifolia Mill. (Lamiaceae) and *Lavandula* × *intermedia* Emeric ex Loisel (Lamiaceae)

The antiparasitic activity of the essential oils of *L. angustifolia* and *L.* × *intermedia* against *G. lamblia* and *T. vaginalis* was investigated. Both essential oils

significantly reduced the viability of the two parasites by causing cell lysis at low concentrations (≤1%). At 0.1%, *L. angustifolia* oil is slightly more effective than *L. x intermedia* oil against *G. lamblia* (duodenalis), so lavender essential oils may be a viable treatment option for parasitic infections (Moon et al., 2006).

ESSENTIAL OILS AGAINST *T. VAGINALIS*

Melaleuca alternifolia Cheel (Myrtaceae)

Commonly known as tea tree oil (TTO), the essential oil is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. The major components are terpinen-4-ol (40.1%), ´Y-terpinene (23.0%), α-terpinene, and 1,8-cineole (5.1%) TTO at 300 mg/ml killed all cells of *T. vaginalis* (Viollon et al., 1996). There is also anecdotal *in vivo* evidence that TTO may be effective in treating *T. vaginalis* infections (Peña, 1962).

ESSENTIAL OILS AGAINST *LEISHMANIA SP*.

Artemisia abrotanum L. (Asteraceae), *Chenopodium ambrosioides* L. (Amaranthaceae), *Pinus caribaea* Morelet (Pinaceae), *Piper aduncum* L. (Piperaceae), and *Piper auritum* Kunt (Piperaceae)

The antiprotozoal activity of *A. abrotanum, C. ambrosoides, P. caribaea, P. adumcum*, and *P. auritum* essential oils against *Leishmania amazonensis* were evaluated. The five oils had MICs similar to that of amphotericin (reference drug), and the lowest MIC was obtained with *C. ambrosoides* essential oil (Monzote et al., 2004). The essential oil of this plant had a potent inhibitory activity against promastigote and amastigote forms, with ED_{50} (50% effective dose) values of 3.7 and 4.6 µg/ml, respectively. The oil showed moderate toxicity in mouse macrophages. At doses of 30 mg/kg/day, the oil was effective against protozoal infection when administered i.p. over 15 days to infected BALB/c mice (Monzote et al., 2006).

Croton cajucara Benth. (Euphorbiaceae)

The essential oil of *C. cajucara* demonstrated activity against *L. amazonensis*. *In vitro* morphological changes in the parasite promastigotes were observed at a concentration of 15 ng/ml of oil, which lead to nuclear and kinetoplast chromatin destruction, followed by cell lysis. Treatment at this concentration to pre-infected murine macrophages caused a 50% reduction in the number of *L. amazonensis* promastigotes infecting macrophages. The main compound of the essential oil is linalool, which has a more potent antileshmanial activity than the essential oil does; LD_{50} for promastigotes and amastigotes are 8.3 and 22 ng/ml for the essential oil and 4.3 and 15.5 ng/ml for linalool, respectively. This oil or its main active component could be of use in the treatment of Leishmaniasis (Rosa et al., 2003).

Achillea millefolium L. (Astereaceae)

The essential oil of leaves and flowers of *A. millefolium* (yarrow) were tested on *L. amazonensis* and murine macrophages. The IC_{50} against *L. amazonensis* promastigotes was 7.8 µg /ml, and the survival of amastigotes of the parasite within peritoneal macrophages was halved by treatment with 6.5 µg/ml of the oil. The mean value for the cytotoxic concentration of the oil, measured against adherent, uninfected J774G8 macrophages, was 72.0 µg/ml. The essential oil caused morphological changes in the treated parasites, including alterations in their shape and size. In transmission electron microscopy, promastigotes treated with the essential oil at the IC_{50} of 7.8 µg/ml showed changes in the flagellar membrane, abnormal membrane structures, rupture of the plasma membrane, atypical vacuoles, myelin-like structures, and vesicles that resembled autophagic vacuoles (Santos et al., 2010).

Cymbopogon citrates DC. (Poaceae)

The inhibitory effect of and the morphological and ultrastructural alterations induced by *C. citrates* essential oil on *L. amazonensis* were evaluated. The activity and effects of the oil's main constituent, citral, was also examined. The antiproliferative activity of the oil on promastigotes, axenic amastigotes, and intracellular amastigotes forms of the parasite were better than those of citral, and its effect was dose-dependent. The essential oil did not show a cytotoxic effect on macrophage strain J774G8. *L. amazonensis* promastigotes underwent morphological and ultrastructural alterations after treatment. These effects were visible by light scanning and by transmission electron microscopy of promastigotes treated with oil or citral at their IC_{50} (1.7 and 8.0 µg/ml) and IC_{90} (3.2 and 25 µg/ml) values, respectively, after 72 h of incubation (Santin et al., 2009).

Lippia sidoides Cham. (Verbenaceae)

The antileishmanial activity of essential oil from *L. sidoides* and of its main constituent thymol was tested on the growth, viability, and ultrastructure of *L. amazonensis*. Both the oil and thymol had activity against the promastigote form of the parasite with IC_{50} values of 44.38 and 19.47 µg/ml, respectively, after 48 h of

incubation. However, thymol showed toxicity on peritoneal macrophages and low selectivity against the promastigotes. In contrast, no cytotoxic effects were observed in macrophages treated with the oil. *L. amazonensis*-infected macrophages incubated with essential oil showed a reduction in amastigotes survival within macrophages. Morphological alterations such as accumulation of large droplets in the cytoplasm, disrupted membrane, and wrinkled cells were seen in treated parasites (Medeiros et al., 2011).

Ocimum gratissimum L. (Lamiaceae)

The leishmanicidal activity of *O. gratissimum* essential oil and its major component, eugenol, was studied. The eugenol-rich essential oil inhibited *L. amazonensis* growth at concentrations from 100 to 1000 µg/ml. The IC_{50} of the oil for promastigotes and amastigotes were 135 and 100 µg/ml, respectively, and the IC_{50} of eugenol was 80 µg/ml for the promastigote form. The parasite exposed to the essential oil at the corresponding IC_{50} for promastigotes and amastigotes underwent considerable ultrastructural alterations.

Two or more nuclei or flagella were observed in 31 and 23.3% of treated amastigotes and promastigotes, respectively. In addition, considerable mitochondrial swelling was observed in promastigotes and amastigotes treated with essential oil, and the inner mitochondrial membranes were altered, with an increase in the number of cristae. The MIC for both forms was 150 µg/ml. Pretreatment of mouse peritoneal macrophages with the essential oil (100 and 150 µg/ml) reduced the indices of association between promastigotes and the macrophages, followed by an increase in nitric oxide production by the infected macrophages (Ueda-Nakamura et al., 2006).

Piper claussenianum (Miq) C. DC. (Piperaceae)

The essential oils from fresh and dried leaves and inflorescences were analyzed, and forty compounds were detected from these oils. Sesquiterpenes were the major components. The activity of the essential oils from fresh leaves and inflorescences were tested against *L. amazonensis*. Both oils showed activity, but only the essential oil from *P. claussenianum* fresh leaves, which was rich in (E)-nerolidol, showed effective growth inhibition of the parasite (Marques et al., 2010).

Artemisia absinthium L. (Astereaceae)

The essential oils of *A. absinthium* and *Echinops kebericho* Mesfin were analyzed by GC/MS. Sixty-five compounds were found for *A. absinthium*; the major constituent was oxygenated monoterpene camphor. The main constituent for *E. kebericho* oil was sesquiterpene lactone dehydrocostus lactone. Both oils showed activity against promastigotes (MIC 0.0097 to 0.1565 µg/ml) and axenic amastigote forms (EC_{50} 0.24 to 42.00 µg/ml) of *Leishmania aethiopica* and *Leishmania donovani*. Weak hemolytic effects were observed for both oils, which showed a slightly decreased selectivity index against the human monocityc leukemia (THP-1) cell line. The oil from *E. kebericho* had stronger antileishmanial activity than the *A. absinthium* oil did (Tariku et al., 2011).

The effect of different essential oils against *Leishmania major* was tested. Of 12 essential oils, only three oils, *Melissa officinalis* L. (Lamiaceae), *T. vulgaris*, and *M. alternifolia* oil, as well as their main compound, terpinen-4-ol, were more toxic to promastigotes of *Leishmania major* than to human HL-60 cells (Mikus et al., 2000).

P. auritum

GC analysis of the essential oil of *P. auritum,* revealed 60 peaks, and safrole was found to be the major component (87%). The oil was active against promastigotes of *L. major*, *Leishmania mexicana, Leishmania brazilensis*, and *L. donovani*. The essential oil inhibited the growth of intracellular amastigotes of *L. donovani*, with an IC_{50} of 22.3 ± 1.8 µg/ml (Monzote et al., 2010).

The essential oils of the three species of Lamiaceae family, *O. basilicum* (sweet basil); (cvs German and Mesten) and *Ocimum sanctum* L. (Holy basil) (syn *Ocimum. tenuiflorum* L.*)* (cv. Local), in Mississipi were studied. The major oil constituents of cvs German and Mester of *O. basilicum* were (-)-linalool (30 to 40%) and eugenol (8 to 30%), whereas the main compounds of cv Local of *O. sanctum* were eugenol (8 to 43%) and methylchavicol (15 to 27%). All the essential oils inhibited the growth of *L. donovani* promastigotes with IC_{50} values in the range of 37 to 50 µg/ml and IC_{90} in the range of 88 to 90 µg/ml. The difference in the oil composition of cultivars did not appear to affect the antileishmanial activity of the basil oils significantly. Minor basil oil constituents (+)-δ-cadinene, 3-carene, α-humulene, citral, and (-)-Trans-caryophyllene had antileishmanial activity, whereas other components were ineffective against the protozoa (Zheljazkov et al., 2008).

The antileishmanial effect of 10 essential oils extracted from 10 plants issued from the Sned region of Tunisia were tested against *L. major* and *L. infantum*, and their cytotoxicity against the murine macrophage cell line RAW 264.7 (ATCC, TIB-71) was also evaluated. The results showed that the oil of *Thymus hirtus* sp. (rich in monoterpenoids, especially linalool and camphor) is active against two species of *Leishmania*, whereas *Ruta chalepensis* oil (rich on 2-undecanone at 84.28%) is only active against *L. infantum*. Both essential oils had low cytotoxicity toward murine macrophages (Ahmed et al., 2011). The antileishmanial activity of 13 essential oils of

different Colombians plants belonging to the families, Lauraceae, Rutaceae, Verbenaceae, Lamiaceae, Zingiberaceae, Myristaceae, Cardiopteridaceae and Pinaceae was tested against *Leishmania brazilensis* promastigotes. The EC_{50} of the essential oils of *Persea caerulea* fruit (Ruiz and Pav) Mez (Lauraceae), *Lippia alba* (Mill.) N.E. Br. ex Britton and P. Wilson (Verbenaceae) leaves, and *Rosmarinus officinalis* L. (Lamiaceae) leaves were 87.8± 55.51, 265.5 ± 7.5, and 17.4 ± 0.43 µg/ml, respectively (Arévalo et al., 2009).

Popularly known as "copaiba," copaiba oil is obtained from plants of the *Copaifera* genus (Fabaceae) and has been used in folk medicine to treat leishmaniasis. The antiproliferative activity of eight different kinds of Brazilian copaiba oil on the promastigotes and amastigotes of *L. amazonensis* were determined. The cytotoxic effect of the oil was assessed in the macrophage strain 1774G8 by assay of sulfohodamine B.

The activity of the copaiba oils against promastigotes is variable, with IC_{50} values in the range between 5 and 22 µg/ml. The most active oil was that from *Copaifera reticulate* with IC_{50} of 5, 15 and 20 µg/ml for promastigote, axenic amastigote and intracellular amastigote forms, and its cytotoxity on macrophages was low (Santos et al., 2008).

Annona foetida Mart (Annonaceae)

The essential oil was obtained by hydrodistillation and analyzed by GC/FID, and the major constituents were found to be bicyclogermacrene (35.12%), (E)-caryophyllene (14.19%), and α-copaene (8.19%). The antileishmanial activity was tested against *L. amazonensis* (MHOM/BR/77/LTB0016), *L. braziliensis* (MHOM/BR/95/IOCL-2033), *L. chagasi* (MCAN/BR/97/P142), and *L. guyanensis* (MHOM/BR/95/IOCL-2092) promastigotes. The best results were obtained against *L. guyanensis*. This oil caused low cytotoxicity to peritoneal macrophages (Costa et al., 2009).

ESSENTIAL OILS AGAINST *TRYPANOSOMA CRUZI*

Origanum vulgare L. (Lamiaceae) and *T. vulgaris*

The effect of the essential oils of *O. vulgare* and *T. vulgaris* on growth and ultrastructure of *T. cruzi* was investigated. The oregano essential oil inhibited epimastigote growth (IC_{50} at 24 h = 175 µg/ml) and induced trypamastigote lysis (IC_{50} at 24 h = 115 µg/ml). The IC_{50} at 24 h of thyme essential oil was 77 µg/ml for epimastigotes and 38 µg/ml for trypomastigotes. Thymol, the main constituent of thyme oil was also tested, the results showed an IC_{50} at 24 h of 62 µg/ml for epimastigotes and 53 µg/ml for tripomastigotes. Scanning electron microscopy of treated cells showed few morphological alterations at the plasma membrane (Santoro et al., 2007).

Nepeta cataria L. (Lamiaceae)

The main compounds of the essential oil of young leaves of *N. cataria* obtained by diethyl ether extraction were α-citral (51.95%), β-citronellol (9.03%), geraniol (4.31%), and nerol (32.24%). The results of the biological test of this oil showed a strong *in vitro* trypanocidal activity against the epimastigotes of *T. cruzi*, with a minimum lethal concentration (MCL, concentration at which all epimastigotes were killed) of 6.2 µM (Saeidnia et al., 2008).

Annona coriaceae Mart. (Annonaceae)

Sixty compounds were found in the essential oil of the leaves from *A. coriaceae,* these compounds were a complex mixture of sesquiterpenes, monoterpenes, and other constituents. Byciclogermacrene was the major component (39.8%), followed by ´Y-muurolene (7.9%), δ-cadinene (6.0%), and (E)-caryophyllene (4.9%), spathulenol (4.2%), α-patchoulene (2.7%), and α humulene (2.4%). The oil presented antileishmanial activity against promastigotes of *L.chagasi, L. amazonensis, L. major*, and *L. brazilensis*. It had a tripanocidal effect on *T. cruzi*, though it proved to be more active against *L. chagasi*, with an IC_{50} of 39.93 µg/ml (95% CI 28.00 to 56.95 µg/ml) (Siqueira et al., 2011).

The chemical composition and biological activities of 19 essential oils of five *Lippia species* (Verbenaceae): *Lippia alba, Lippia citriodora*, *Lippia micromera* Schauer*, Lippia origanoides* Kunth, and *Lippia dulcis* Trevir, which were collected in different places in Colombia were examined. The main components of the oils were geranial, neral, limonene, nerol, carvacrol, ρ-cimene, ´Y-terpinene, S-carvone, and thymol. The highest activity was shown by *L. alba* against *T. cruzi* epimastigotes and intracellular amastigotes, with an IC_{50} of 5.5 and 12.2 µg/ml, respectively. The essential oil of *L. origanoides* had an IC_{50} of 4.4 µg/ml against *L. chagasi* promastigotes and showed no toxicity in mammalian cells. Thymol (IC_{50} 3.2 ± 0.4 µg/ml) and S-carvone (IC_{50} 6.1 ± 2.2 µg/ml), two of the major components of the essential oils, were active against intracellular amastigotes of *T. cruzi*-infected Vero cells with a selective index greater than 10 (Escobar et al., 2010).

DISCUSSION

Infections caused by parasites protozoa are of great importance in public health because of its high prevalence, distribution and its effects on the population. The drugs currently used to treat these diseases have serious side effects, so it is relevant to look for new pharmacological alternatives. Pharmaceutical research

on natural products represents a strategy for discovering and developing new drugs. However, little work has been carried out using purified essential oils. Essential oils (EO) are very complex natural mixtures which can contain about 20 to 60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20 to 70%) (Bakkali et al., 2008).

Generally, these major components determine the biological properties of the essencial oils (Pichersky et al., 2006). The components include two groups of the distinct biosynthetical origin. Approximately 3000 essential oils are known, 300 of which are commercially importance especially for the pharmaceutical, agronomic, food, cosmetic and perfume industries. EO possess antibacterial, antifungal, antiviral and antiseptic properties; they are used as antimicrobial, analgesic, sedative, antiinflamatory, spasmolytic and locally anesthesic remedies. Also, they are used for preservation of food and insecticide (Bakkali et al., 2008). Several essential oil such as oils of garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, laurel, ginger, rosemary, and peppermint, among others, have been studied as antimicrobial natural products against both bacteria and molds.

Plant essential oils and active components can be used as alternatives or additions to current antiparasitic therapies. The present review discussed the activity of essential oils from 42 plant species, which belong to 15 families. Of these plants, 16 species were from Lamiaceae, 7 from Verbenaceae, 4 from Asteraceae, 3 from Piperaceae, and 2 were from Anonaceae; the other 10 families are represented with one species each. In the Lamiaceae family, the *Origanum* genus was the most abundant; in the Verbenaceae, only the *Lippia* genus appears; in Asteraceae, *Artemisia* genus was the most frequent; for Piperaceae, all of the species were from the *Piper* genus; and with the Annonaceae, both the species were from the *Annona* genus.

The following constituents were the most commonly found compounds in essential oils with antiprotozoal activity: Thymol, which appears in *L. alba, L. ciriodora, L. dulcis, L. micromera, L. origanoides, O. basilicum*, and *T. vulgaris*; eugenol, which was found in *O. basilicum, O. gratissimum. O. sanctum*, and *S. aromaticum*; camphor, which was present in *A. absinthium* and *T. hirtus*; carvacrol, which was a constituent of *L. alba*, *O. virens*, and *T. capitata*; and terpinen-4-ol, which was a component of *M. alternifolia, M. officinalis* and *T. vulgaris*. The essential oils we have reviewed may represent a step forward in the search for novel antiparasitic agents, helping to accelerate the development of effective and safe treatments for various protozoal diseases.

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