Antiprotozoal Activity of Essential Oils

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Summary

In the present scenario of protozoal infections, new drugs are urgently needed to treat and control infections such as malaria, sleeping sickness, Chagas disease, leishmaniasis and intestinal infections, which affect millions of people each year. In this review, we are focusing on articles related to antiprotozoal essential oils extracted from plants that have been published during the last 20 years. The data analyzed indicate that essential oils could be promising antiprotozoal agents, opening perspectives to the discovery of more effective drugs of vegetal origin for the treatment of diseases caused by protozoa.

Key words

essential oils, parasite, *Leishmania*, *Plasmodium*, *Trypanosoma*, *Giardia*, *Trichomonas*

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Introduction

Essential oils (EOs) are volatile extracts obtained through steam distillation of aromatic plants (Post-White and Nicholson, 2007). The aromatic oily liquids can be obtained from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots, commonly extracted by distillation using a Clevenger's apparatus (Burt, 2004). There are an estimated 3000 known EOs about 300 of which are commercially important destined chiefly for the flavour and fragrances market (Van de Braak and Leijten, 1999).

The medicinal values of EOs have been utilized in various forms. The antimicrobial actions of EOs are one of the most extensively studied aspects of botanical medicine. EOs has been shown to exhibit antibacterial (Burt, 2004), antiviral (Edris, 2007), antimycotic (Pisseri *et al.*, 2008) and antiparasitic activity (Antony *et al.*, 2005). These characteristics are possibly related to the presence of diverse compounds in the EOs (Mahmoud and Croteau, 2002). The purpose of this paper is to provide an overview of the published data about antiparasitic activity of EOs and their components, with special reference to parasitic infections in humans.

With the objective to obtain the reports about antiparasitic potentialities of EOs, an electronic search was performed from 1988 to the present. We have consulted "Pubmed" database, which is one of the most used by academics, medical students and primary care practitioners according to several surveys (Cullen, 2002; DeGroote and Dorsch, 2003; Tannery *et al.*, 2002). In parallel, journals specifically dedicated to EO research were also reviewed (*Journal of Essential Oil Research*, *Flavour and Fragrance Journal*, *International Journal of Essential Oil Therapeutics,* and *Journal of Essential Oil-Bearing Plants*). The search was performed using the keyword essential oil combined with: parasite, *Plasmodium, Trypanosoma, Leishmania, Toxoplasma* and *Trichomonas,* respectively. The reports related to antiprotozoal activity of EOs were analyzed. Those reports concerning animal parasites and plants/agriculture parasites were excluded.

Properties and Components of Antiprotozoal Essential Oils

Figure 1 shows an increase in publications related to anti parasitic protozoal essential oils, indicating a steady increase in scientific research and interest during the last few years. A compilation of antiparasitic essential oils is presented in Table 1. *Cymbopogon citratus* and *Ocimum gratissimum* were the plants most investigated against *Plasmodium, Trypanosoma* and *Leishmania* parasites. The second most cited plants were *Ocimum basilicum, Origanum vulgare* and *Thymus vulgaris* studied against *Trypanosoma cruzi* and intestinal parasites. A correlation between the number of reports and the diseases that cause higher morbidity and mortality can be observed: 22 reports about *Plasmodium*, 14 against *Trypanosoma*, 26 against *Leishmania*, three against *Trichomonas*, and four intestinal protozoa.

The active components of EOs can be isolated by HPLC and gas liquid chromatography and characterized by different detectors (mainly mass spectrometry). Major components can constitute up to 85% of the EO, whereas other components are present only in trace amounts (Bauer *et al.*, 1985). The composition of EOs from a particular species of plant can differ between harvesting seasons, geographical sources and parts of the same plant. Nevertheless, EOs produced from herbs harvested during or immediately after flowering generally possesses the strongest activity (Burt, 2004). The main constituents of the EOs with antiprotozoal activity are monoterpenoids (linalool, terpinen-4-ol, thymol, carvacrol, citral, limonene, α-pinene, γ-terpinene, α-phellandrene, and *p*-cymene), sesquiterpenes (β-caryophyllene, nerolidol, α-copaene, cyperene, and germacrene D) and phenylpropanoids (eugenol, methyl chavicol, and cinnamaldehyde), which are responsible of their biological effects. A list of antiparasitic essential oil components is presented in Table 2.

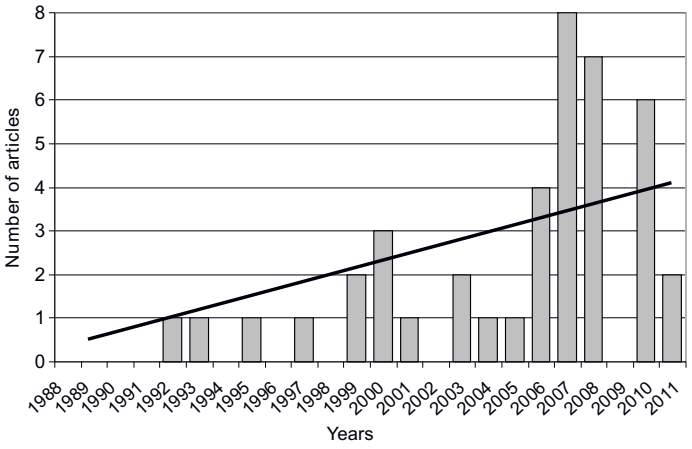

Figure 1. Number of articles found in MEDLINE about human antiparasitic EO from plants by year

Antiparasitic Effects of Plant-Derived EOs

Antiplasmodial Activity. The Amazonian Indians from Brazil treat malaria with an inhalation of vapor obtained from leaves of *Virola surinamensis*. The EOs obtained from adult and plantlet leaves (rich in α-pinene, *p*-cymene, and (*E*)-nerolidol) had caused 100% of growth inhibition after 48 h in the development of the young trophozoite to schizont stage of *Plasmodium falciparum* (Lopes *et al.*, 1999). Nerolidol was identified in this work as one of the active constituents, which was later reconfirmed by Rodrigues Goulart and co-workers, demonstrating a median inhibitory concentration (IC_{50}) of 760 nM (Rodrigues *et al.*, 2004). Farnesol was also identified as an antiplasmodial sesquiterpene alcohol (Lopes *et al.*, 1999).

The EO of *Lippia multiflora* is another nerolidol-rich antiplasmodial EO. It was tested on *in vitro* cultures of *P. falciparum* (chloroquine-sensitive and resistant strains). The EO inhibited the growth of parasites and when tested on a highly synchronized culture, the oil inhibited growth mostly at the trophozoite-schizont step, indicating their potential effect (Valentin *et al.*, 1995).

Five EOs extracted from the Cameroonian plants (*Antidesma laciniatum*, *Hexalobus crispiflorus, Pachypodanthium confine, Xylopia aethiopica* and *Xylopia phloiodora*) were evaluated in regard to their anti-plasmodial effect. The oils were active against *P. falciparum* in culture and the most effective one was the oil

Table 1. Plant-derived essential oils that showed antiparasitic activity (1988-present)

Plant Essential Oil	Family	Parasitic organism	Reference
Achillea millefolium L.	Asteraceae	*Leishmania amazonensis* *Trypanosoma cruzi*	Santos *et al.*, 2010 Santoro *et al.*, 2007a
Aframomum sceptrum (Oliv. & T. Hanb.) K. Schum.	Zingiberaceae	*Trypanosoma brucei* *Trichomonas vaginalis*	Cheikh-Ali *et al.*, 2011
Ambrosia scabra Hook. & Arn.	Asteraceae	*Plasmodium falciparum*	Sülsen *et al.*, 2008
Ambrosia tenuifolia Spreng.	Asteraceae	*Plasmodium falciparum* *Trypanosoma cruzi*	Sülsen *et al.*, 2008
Annona foetida Mart.	Annonaceae	*Leishmania guyanensis*	Costa *et al.*, 2009
Antidesma laciniatum Müll. Arg.	Phyllanthaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003
Artemisia abyssinica Sch. Bip.	Asteraceae	*Leishmania donovani* *Leishmania aethiopica*	Tariku *et al.*, 2010
Artemisia gorgonum Webb	Asteraceae	*Plasmodium falciparum*	Ortet *et al.*, 2010
Artemisia herba-alba Asso.	Asteraceae	*Leishmania major* *Leishmania tropica*	Hatimi *et al.*, 2001
Baccharis dracunculifolia DC.	Asteraceae	*Leishmania donovani*	Parreira *et al.*, 2010
Chenopodium ambrosioides L.	Chenopodiaceae	*Leishmania amazonensis* *Leishmania donovani*	Monzote *et al.*, 2006 Monzote *et al.*, 2007a
Cochlospermum planchonii Hook. f. ex Planch.	Cochlospermaceae	*Plasmodium falciparum*	Benoit-Vical *et al.*, 1999
Cochlospermum tinctorium A. Rich.	Cochlospermaceae	*Plasmodium falciparum*	Benoit-Vical *et al.*, 1999
Copaifera cearensis Huber ex Ducke	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera langsdorfii Desf.	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera lucens Dwyer	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera martii Hayne	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera multijuga Hayne	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera officinalis (Jacq.) L.	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera paupera (Herzog) Dwyer	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Copaifera reticulata Ducke	Fabaceae	*Leishmania amazonensis*	Santos *et al.*, 2008
Croton cajucara Benth.	Euphorbiaceae	*Leishmania amazonensis*	Rosa *et al.*, 2003
Cymbopogon citratus (DC.) Stapf.	Poaceae	*Crithidia deanei*[a] *Leishmania amazonensis* *Leishmania chagasi* *Leishmania infantum* *Plasmodium berghei*[a] *Trypanosoma cruzi*	Pedroso *et al.*, 2006 Santin, *et al.*, 2009 Oliveira *et al.*, 2009 Machado *et al.*, 2010b Tchoumbougnang *et al.*, 2005 Santoro *et al.*, 2007a
Daucus crinitus Desf.	Apiaceae	*Plasmodium falciparum*	Lanfranchi *et al.*, 2010
Hagenia abyssinica J.F. Gmel.	Rosaceae	*Trypanosoma brucei*[a]	Nibret and Wink, 2010
Helichrysum cymosum (L.) Less.	Asteraceae	*Plasmodium falciparum*	van Vuuren *et al.*, 2006
Hexalobus crispiflorus A. Rich.	Annonaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003
Juniperus oxycedrus L.	Cupressaceae	*Leishmania infantum*	Machado *et al.*, 2010b
Lavandula angustifolia Mill.	Lamiaceae	*Giardia duodenalis* *Trichomonas vaginalis*	Moon *et al.*, 2006
Lavandula × *intermedia* Emeric ex Loisel.	Lamiaceae	*Giardia duodenalis* *Trichomonas vaginalis*	Moon *et al.*, 2006
Leonotis ocymifolia (Burm. f.) Iwarsson	Lamiaceae	*Trypanosoma brucei*[a]	Nibret and Wink, 2010
Licaria cannella (Meisn.) Kosterm.	Lauraceae	*Leishmania amazonensis*	Silva *et al.*, 2009
Lippia alba (Mill.) N.E. Br. ex Britton & P. Wilson	Verbenaceae	*Leishmania chagasi* *Trypanosoma cruzi*	Escobar *et al.*, 2010
Lippia citrodora Kunth	Verbenaceae	*Leishmania chagasi* *Trypanosoma cruzi*	Escobar *et al.*, 2010
Lippia dulcis Trevir.	Verbenaceae	*Leishmania chagasi* *Trypanosoma cruzi*	Escobar *et al.*, 2010
Lippia graveolens Kunth	Verbenaceae	*Giardia lamblia*	Machado *et al.*, 2010a
Lippia javanica (Burm f.) Spreng.	Verbenaceae	*Plasmodium falciparum*	Manenzhe *et al.*, 2004
Lippia micromera Schauer	Verbenaceae	*Leishmania chagasi* *Trypanosoma cruzi*	Escobar *et al.*, 2010
Lippia multiflora Moldenke	Verbenaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003
Lippia origanoides Kunth	Verbenaceae	*Leishmania chagasi* *Trypanosoma cruzi*	Escobar *et al.*, 2010
Lippia sidoides Cham.	Verbenaceae	*Leishmania chagasi*	Oliveira *et al.*, 2009
Melaleuca alternifolia Cheel	Myrtaceae	*Leishmania major* *Trypanosoma brucei*[a]	Mikus *et al.*, 2000
Melissa officinalis L.	Lamiaceae	*Leishmania major* *Trypanosoma brucei*[a]	Mikus *et al.*, 2000
Moringa stenopetala (Baker f.) Cufod.	Moringaceae	*Trypanosoma brucei*[a]	Nibret and Wink, 2010
Myoporum crassifolium Forster & G. Forster	Myoporaceae	*Leishmania amazonensis*	Billo *et al.*, 2005
Myrtus communis L.	Myrtaceae	*Plasmodium falciparum*	Milhau *et al.*, 1997
Ocimum basilicum L.	Lamiaceae	*Giardia lamblia* *Leishmania donovani* *Trypanosoma cruzi*	de Almeida *et al.*, 2007 Zheljazkov *et al.*, 2008 Hoet *et al.*, 2006

Table 1 - continued

Ocimum gratissimum L.	Lamiaceae	*Herpetomonas samuelpessoai*[a] *Leishmania amazonensis* *Leishmania chagasi* *Plasmodium berghei*[a]	Holetz *et al.*, 2003 Ueda-Nakamura *et al.*, 2006 Oliveira *et al.*, 2009 Tchoumbougnang *et al.*, 2005
Ocimum sanctum L.	Lamiaceae	*Leishmania donovani*	Zheljazkov *et al.*, 2008
Origanum virens L.	Lamiaceae	*Giardia lamblia*	Machado *et al.*, 2010a
Origanum vulgare L.	Lamiaceae	*Blastocystis hominis* *Endolimax nana* *Entamoeba hartmanni* *Trypanosoma cruzi*	Force *et al.*, 2000 Force *et al.*, 2000 Force *et al.*, 2000 Santoro *et al.*, 2007c
Pachypodanthium confine Engl. & Diels	Annonaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003
Piper auritum Kunth	Piperaceae	*Leishmania braziliensis* *Leishmania donovani* *Leishmania major* *Leishmania mexicana*	Monzote *et al.*, 2010
Piper claussenianum (Miq.) C. DC.	Piperaceae	*Leishmania amazonensis*	Marques *et al.*, 2010
Rosmarinus officinalis L.	Lamiaceae	*Plasmodium falciparum*	Milhau *et al.*, 1997
Salvia albicaulis Benth.	Lamiaceae	*Plasmodium falciparum*	Kamatou *et al.*, 2007
Salvia dolomitica Codd	Lamiaceae	*Plasmodium falciparum*	Kamatou *et al.*, 2007
Satureja punctata (Benth.) Briq.	Lamiaceae	*Leishmania donovani* *Leishmania aethiopica*	Tariku *et al.*, 2010
Strychnos spinosa Lam.	Loganiaceae	*Trypanosoma brucei*[a]	Hoet *et al.*, 2006
Syzigium aromaticum L.	Myrtaceae	*Trypanosoma cruzi* *Giardia lamblia*	Santoro *et al.*, 2007b Machado *et al.*, 2011
Tetradenia riparia (Hochst.) Codd	Lamiaceae	*Plasmodium falciparum*	Campbell *et al.*, 1997
Thymbra capitata Cav.	Lamiaceae	*Giardia lamblia*	Machado *et al.*, 2010a
Thymus capitellatus Hoffmanns. & Link	Lamiaceae	*Leishmania infantum*	Machado *et al.*, 2010b
Thymus vulgaris L.	Lamiaceae	*Entamoeba histolytica* *Leishmania major* *Trypanosoma brucei*[a] *Trypanosoma cruzi*	Behnia *et al.*, 2008 Mikus *et al.*, 2000 Mikus *et al.*, 2000 Santoro *et al.*, 2007c
Thymus zygis L.	Lamiaceae	*Giardia lamblia*	Machado *et al.*, 2010a
Virola surinamensis (Rol. ex Rottb.) Warb.	Myristicaceae	*Plasmodium falciparum*	Lopes *et al.*, 1999
Xylopia aethiopica (Dunal) A. Rich.	Annonaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003
Xylopia phloiodora Mildbr.	Annonaceae	*Plasmodium falciparum*	Boyom *et al.*, 2003

[a] As an experimental model organism.

extracted from *H. crispiflorus* (dominated by the sesquiterpenes α-copaene, cyperene, alloaromadendrene, δ-cadinene, calacorene, and τ-cadinol), at a IC_{50} of 2 µg/mL (Boyom *et al.*, 2003).

The antimalarial and toxicological properties of *Cochlospermum tinctorium* and *C. planchonii* EOs prepared from their leaves were studied. After 24 and 72 h contact between the oils and the parasite culture, *C. planchonii* EO (rich in sesquiterpene hydrocarbons β-caryophyllene and (*E*,*E*)-α-farnesene) showed an IC_{50} of 22-35 µg/mL against *P. falciparum*. The cytotoxicity of oils was assessed on the K562 cell line and showed IC_{50} values ranging between 33 and 2000 g/mL (Benoit-Vical *et al.*, 1999).

Antitrypanosomal Activity. The anti-proliferative effect of *Cymbopogon citratus* (lemongrass) oil was demonstrated against *Trypanosoma cruzi*. The IC_{50} of the oil against free cells of the epimastigote and trypomastigote forms was 126.5 and 15.5 µg/mL, respectively, after 24 h of treatment. An IC_{50} of 5.1 µg/mL after 48 h of treatment of intracellular amastigote in mouse peritoneal macrophage was determined. *C. citratus* oil is rich in citral (a mixture of geranial and neral) and this was shown to be responsible for the trypanocidal activity (Santoro *et al.*, 2007a).

The trypanocidal activities of clove (*Syzigium aromaticum*), basil (*Ocimum basilicum*) and yarrow (*Achillea millefolium*) essential oils were determined on *T. cruzi* epimastigote and bloodstream trypomastigote forms. The clove oil (dominated by eugenol) was found to be the most effective, with an IC_{50} value of 99.5 µg/mL for epimastigotes and 57.5 µg/mL for trypomastigotes (Santoro *et al.*, 2007b).

Strychnos spinosa is used in African traditional medicine to treat African trypanosomiasis. The EO obtained from the leaves was evaluated against *Trypanosoma brucei brucei* bloodstream forms, as a model to predict antitrypanosomal activity, and on mammalian cells (J774 murine macrophages) to evaluate the selectivity of the antitrypanosomal effect. The EO was active on the parasites; showing an IC_{50} of 13.5 µg/mL with a selectivity index of 4.4. The bioactive agents were determined to be linalool (IC_{50} = 2.5 µg/mL) and nerolidol (IC_{50} = 1.7 µg/mL), with a selectivity index higher than 30 (Hoet *et al.*, 2006). The activities of these components on *T. brucei brucei* have very recently been confirmed (Nibret and Wink, 2010).

Aframomum sceptrum is found in humid forest regions of tropical Central Africa and has been used in traditional medicine as an antiparasitic in Ivory Coast (Okpekon *et al.*, 2004) and Nigeria (Idu and Osemwegie, 2007). The EO from the rhizome of *A. sceptrum* has shown notable *in-vitro* antiparasitic activity on *T. brucei brucei* with a minimum lethal concentration (MLC) of 1.51 µL/mL, which may be attributable to a high concentration of caryophyllene oxide (10%); the MLC of caryophyllene oxide on *T. brucei brucei* was determined to be 0.1 mg/mL (Cheikh-Ali *et al.*, 2011).

Table 2. Essential oil components known to exhibit antiparasitic activity

Compound	Chemical class	Parasitic organism	Reference
Alloaromadendrene	Sesquiterpene hydrocarbon	*Trypanosoma brucei*	Mikus *et al.*, 2000
Aromadendrene	Sesquiterpene hydrocarbon	*Leishmania major*	Mikus *et al.*, 2000
Asarone	Phenylpropanoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Benzyl isothiocyanate	Isothiocyanate	*Trypanosoma brucei*	Nibret and Wink, 2010
α-Bisabolol	Sesquiterpenoid	*Leishmania infantum*	Morales-Yuste *et al.*, 2010
δ-Cadinene	Sesquiterpene hydrocarbon	*Leishmania donovani*	Santos *et al.*, 2008
Camphor	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
δ-3-Carene	Monoterpene hydrocarbon	*Leishmania donovani*	Santos *et al.*, 2008
Carvacrol	Aromatic monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Carvone	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
β-Caryophyllene	Sesquiterpene hydrocarbon	*Leishmania donovani* *Trypanosoma brucei*	Santos *et al.*, 2008 Nibret and Wink, 2010
Caryophyllene oxide	Sesquiterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
α-Cedrene	Sesquiterpene hydrocarbon	*Trypanosoma brucei*	Nibret and Wink, 2010
Cinnamaldehyde	Phenylpropanoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Citral (mixture of geranial and neral)	Monoterpenoid	*Leishmania amazonensis* *Leishmania donovani* *Trypanosoma cruzi*	Santin *et al.*, 2009 Santos *et al.*, 2008 Santoro *et al.*, 2007a
Citronellal	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Estragole	Phenylpropanoid	*Trypanosoma brucei brucei*	Nibret and Wink, 2010
Eugenol	Phenylpropanoid	*Giardia lamblia* *Leishmania amazonensis* *Trypanosoma brucei*	de Almeida *et al.*, 2007 Ueda-Nakamura *et al.*, 2006 Nibret and Wink, 2010
Farnesol	Sesquiterpenoid	*Plasmodium falciparum*	Rodrigues *et al.*, 2004
Germacrene D	Sesquiterpene hydrocarbon	*Trypanosoma cruzi*	Arruda *et al.*, 2005
α-Humulene	Sesquiterpene hydrocarbon	*Leishmania donovani*	Santos *et al.*, 2008
Limonene oxide	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Linalool	Monoterpenoid	*Giardia lamblia* *Plasmodium falciparum* *Trypanosoma brucei*	de Almeida *et al.*, 2007 Rodrigues *et al.*, 2004 Hoet *et al.*, 2006; Nibret and Wink, 2010
Myrtenal	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
(*E*/*Z*)-Nerolidol	Sesquiterpenoid	*Leishmania amazonensis* *Plasmodium falciparum* *Trypanosoma brucei*	Arruda *et al.*, 2005 Lopes *et al.*, 1999: Rodrigues *et al.*, 2004 Hoet *et al.*, 2006
(*Z*)-Nerolidol	Sesquiterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Oplopanone	Sesquiterpenoid	*Trypanosoma cruzi*	Biavatti *et al.*, 2001
α-Phellandrene	Sesquiterpene hydrocarbon	*Leishmania major* *Trypanosoma brucei*	Mikus *et al.*, 2000
α-Pinene	Monoterpene hydrocarbon	*Leishmania major* *Trypanosoma brucei*	Mikus *et al.*, 2000
Piperitone	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Sabinene	Monoterpene hydrocarbon	*Trypanosoma brucei*	Mikus *et al.*, 2000
Safrole	Phenylpropanoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Terpinen-4-ol	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010 Mikus *et al.*, 2000
Thujone	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010
Thymol	Aromatic monoterpenoid	*Trypanosoma brucei* *Trypanosoma cruzi*	Nibret and Wink, 2010 Santoro *et al.*, 2007c
Verbenone	Monoterpenoid	*Trypanosoma brucei*	Nibret and Wink, 2010

A number of *Lippia* spp. (Verbenaceae) from Colombia were screened for antitrypanosomal activity against *T. cruzi*, and those samples that were rich in either neral, geranial, geraniol, thymol, or carvacrol were particularly active (Escobar *et al.*, 2010). These workers also showed that geranial, carvacrol, and thymol exhibited antitrypanosomal activity.

Antileishmanial Activity. A linalool-rich EO from leaves of *Croton cujacara* has been used successfully against *Leishmania* parasite. The oil showed an IC_{50} of 8.3 ng/mL and 22 ng/mL against promastigotes and amastigotes of *Leishmania amazonensis* and no toxic effects on mammalian cells were observed (Rosa *et al.*, 2003).

The eugenol-rich essential oil of *Ocimum gratissimum* progressively inhibited *L. amazonensis* growth at concentrations ranging from 100 to 1000 µg/mL. The IC_{50} of this oil against promastigotes and amastigotes was 135 and 100 µg/mL, respectively. The product showed no cytototoxic effects against mammalian cells (Ueda-Nakamura *et al.*, 2006). Both *Ocimum basilicum* and *O. sanctum* (both also rich in eugenol) showed antileishmanial activity on *L. donovani* promastigotes, but were devoid of cytotoxicity on kidney fibroblast or kidney epithelial cells (Zheljazkov *et al.*, 2008). Eugenol, a phenylpropanoid extracted from *O. gratissimum,* showed an IC_{50} of 80 µg/mL against promastigote forms of *L. amazonensis* (Ueda-Nakamura *et al.*, 2006).

The use of copaiba oils to treat leishmaniasis is cited in several ethnopharmacological studies. Eight different kinds of Brazilian copaiba oils (*Copaifera cearensis, C. langsdor, C. lucens, C. martii, C. multijuga, C. officinalis, C. paupera* and *C. reticulata*) were screened for antileishmanial activity (Santos *et al*., 2008). Copaiba oils showed variable levels of activity against promastigote forms with IC_{50} values in the range between 5 and 22 µg/mL. The most active EOs were from *C. reticulata* and *C. multijuga* (both rich in β-caryophyllene). The cytotoxicity of *C. reticulata* EO was low against J774G8 macrophages.

Chenopodium oil showed an IC_{50} against promastigotes and amastigotes of *L. amazonensis* of 3.7 and 4.6 µg/mL, respectively (Monzote *et al*., 2006); while against *L. donovani* was of 4.5 and 5.1 µg/mL (Monzote *et al*., 2007a). The EO was effective by intraperitoneal and oral routes when BALB/c mice experimentally infected with *L. amazonensis* were treated (Monzote *et al*., 2007b). A synergic action has been observed in conjunction with the pentamidine, drug clinically used, in promastigotes growing freely in Schneider's medium (Monzote *et al*., 2007c).

The EO from *Artemisia herba-alba* was tested for antileishmanial activity against *L. tropica* and *L. major*, and showed leishmanicidal activity of 2 µg/mL against the two organisms (Hatimi *et al*., 2001). Similarly, *A. abyssinica* EO was active against *L. donovani* and *L. aethiopica* (Tariku *et al*., 2010).

Activity against Intestinal Protozoa. The effect of *Ocimum basilicum* EO on *Giardia lamblia* was studied *in vitro*. Pretreatment of peritoneal mouse macrophages with 2 mg/mL EOs dilution reduced in 79% the association index between these macrophages and *G. lamblia*, with a concomitant increase by 153% on nitric oxide production by the *G. lamblia*-ingested macrophages. One of the major components of *O. basilicum* EO was linalool, which was able to kill 100% of *G. lamblia* parasites after 1 h of incubation at 300 µg/mL (de Almeida *et al*., 2007).

Two EOs derived from *Lavandula angustifolia* and *L.* × *intermedia* were investigated for antiparasitic activity against the human protozoal pathogens *Giardia lamblia*, *Trichomonas vaginalis*, and *Hexamita inflata*. This study has demonstrated that low (≤1%) concentrations of *L. angustifolia* and *L.* × *intermedia* oil can completely eliminate *G. lamblia* in culture. At 0.1% concentration, *L. angustifolia* oil was found to be slightly more effective than *L.* × *intermedia* oil against *G. lamblia* (Moon *et al*., 2006). These *Lavandula* EOs are both dominated by linalool (Lane and Mahmoud, 2008), which may be responsible for the observed activity.

The EO isolated from *Thymus vulgaris* of Iranian origin was active against the trophozoites of *E. histolytica*, which was more effective than hydroalcoholic and hexane extracts (Behnia *et al*., 2008). *T. vulgaris* oil is dominated by thymol (Stoilova *et al*., 2008) and this phenolic component is likely responsible for the activity. A recent report by Machado *et al*. (2010a) has shown essential oils rich in phenolic compounds to exhibit antigiardial activity.

Oil of Mediterranean oregano, *Origanum vulgare*, was orally administered to 14 adult patients whose stools tested positive for enteric protozoa: *Blastocystis hominis, Entamoeba hartmanni* and *Endolimax nana*. After six weeks of supplementation with 600 mg emulsified oil of oregano daily, there was complete disappearance of *E. hartmanni* (four cases), *E. nana* (one case), and *B. hominis* (eight cases). In addition, *Blastocystis hominis* scores declined in three additional cases. Gastrointestinal symptoms improved in seven of the 11 patients who had tested positive for *Blastocystis hominis* (Force *et al*., 2000). Note that *O. vulgare* oil, like *T. vulgaris* oil, is rich in thymol as well as carvacrol (D'Antuono *et al*., 2000).

Mechanism of Antiparasitic Activity of EOs

An important feature of the EOs and their components is their hydrophobicity and low density that can enhance the targeting of active components within the oil to intracellular parasites (Boyom *et al*., 2003). It might also offer alternative delivery routes, including transcutaneous delivery following scarification or patch application (Antony *et al*., 2005).

Two mechanisms of action can be attributed to the efficacy of plant EOs: their direct antiparasitic action and their immunomodulatory properties (Antony *et al*., 2005). The mechanism of action of the EOs has not been studied in great detail and there are large numbers of chemical compounds present in the oils. It is likely that the pharmacologic action would not be attributable to one specific mechanism but to several targets in the cell (Valentin *et al*., 1995). In addition, the inherent activity of the oil can be expected to relate their chemical compositions. That is, the proportions in which they are present and the interactions between them are responsible for their pharmacological activity.

Direct Antiparasitic Activity. In most of the scientific articles only ultrastructural studies have been reported [see (Santoro *et al*., 2007c; Oliveira *et al*., 2009; Santin *et al*., 2009; Adade and Souto-Padrón, 2010), for example]. These studies are important in compounds without previous knowledge about their mechanism of action, in determining their targets in the parasites. Considerable mitochondrial swelling was observed in EOs from *O. gratissimum*-treated *L. amazonensis* promastigotes and amastigotes, which had the inner mitochondrial membrane altered, with a significant increase in the number of cristae; in some amastigotes the mitochondrial matrix became less electrondense (Ueda-Nakamura *et al*., 2006).

Other mechanisms of action have been suggested. The EO from *O. basilicum* (2 mg/mL) as well as purified linalool (300 µg/mL) showed protease inhibitory activity, mainly of cysteine proteases (de Almeida *et al*., 2007). A number of essential oils (Setzer *et al*., 2007a; Setzer *et al*., 2007b; Stokes *et al*., 2007) and essential oil components (Setzer *et al*., 2007b) have shown inhibitory activity against the cysteine protease cruzain, presumably due to binding of these hydrophobic agents to the hydrophobic recognition site of the enzyme (Ogungbe and Setzer, 2008). The EOs from *V. surinamensis* and nerolidol have been shown to inhibit glycoprotein biosynthesis by competing with the biosynthesis of isoprenoid derivatives (Lopes *et al*., 1999; Rodrigues *et al*., 2004).

Immunostimulatory Activity of EOs. The diverse structural and biochemical properties, their life cycles, and pathogenic mechanisms of protozoal parasites has led to the different and complex immune responses by vertebrate hosts, including humans and animals. Most protozoal infections are chronic because of weak innate immunity and ability of parasite to evade or resist elimination by an adaptive immune response. Furthermore, many antiparasitic drugs are not effective at killing the organ-

ism. Individuals living in endemic areas require repeated chemotherapy because of continued exposure, and such treatment is often not possible due to cost and logistical problems. The development of prophylactic vaccines for parasites has long been considered and important goal for developing countries (Costa *et al.*, 2009). However, the progress in the development of vaccines against protozoal infections tends to be slow and arduous (Parreira *et al.*, 2010). In this scenario, immunotherapy has been adopted as a new situation together with antiprotozoal agents.

The immunomodulatory effects of some EOs have demonstrated their usefulness for treating infectious diseases (Antony *et al.*, 2005). The EO from *C. cajucara* and *C. ambrosioides* enhanced macrophage nitric oxide production, which is a potent intracellular parasite-killing mechanism in macrophages (Rosa *et al.*, 2003; Pedroso *et al.*, 2006). The anti-inflammatory activity of EOs has been also reported, which contribute to protecting the host from the damaging effects of the inflammatory components of the immune response as they control the parasitic infections (Antony *et al.*, 2005). *Melaleuca alternifolia* EO caused the stimulation of lymphocytes and increased the secretion of the anti-inflammatory cytokines (Machado *et al.*, 2010a), while *O. sanctum* oil demonstrated anti-inflammatory activity due to dual inhibition of arachidonate metabolism (Tchoumbougnang *et al.*, 2005).

Nigella sativa seed oil has been found to reduce parasitemia and to extend the life span of *T. brucei* infected rats (Ekanem *et al.*, 2008). *N. sativa* seed oil treatment resulted in increased hemoglobin concentration, increased red blood cell, white blood cell, and platelet counts, suggesting that the oil has trypanocidal properties as well as host immune stimulant activity.

Conclusion

In this review, we presented an overview of the different uses of EOs against parasitic protozoa. One of the most interesting areas of application for EOs is the inhibition of growth and reduction of parasite burden. The data collected indicate that EOs can serve as promising antiprotozoal agents, opening perspectives to the discovery of new alternatives drugs of vegetal origin for treatment of protozoal diseases. Nevertheless, the main results about potentialities of EOs have been demonstrated in *in-vitro* studies. Pharmacological tests in *in-vivo* models are needed to confirm the therapeutic potential of essential oils against parasites. Additional toxicity evaluations must be performed, together with the elucidation of these mechanisms would provide insights that may prove useful for technological applications. The international standardisation of EOs can help to advances these natural products as commercially available and efficacious new drugs to combat protozoal diseases. We hope that this paper stimulates interest for researches of the potentialities of EOs as antiprotozoal drugs.

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