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Original Article

Chemical composition, antischistosomal and cytotoxic effects of the essential oil of *Lavandula angustifolia* grown in Southeastern Brazil

André L.L. Mantovani[a], *Geovana P.G. Vieira*[a], *Wilson R. Cunha*[a], *Milton Groppo*[b], *Raquel A. Santos*[a], *Vanderlei Rodrigues*[c], *Lizandra G. Magalhães*[a], *Antônio E.M. Crotti*[a,d,*]

[a]*Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, Franca, SP, Brazil*
[b]*Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil*
[c]*Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil*
[d]*Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil*

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ABSTRACT

This paper reports on the chemical composition, the *in vitro* antischistosomal effects, and the cytotoxicity of the essential oil from the leaves of *Lavandula angustifolia* Mill., Lamiaceae, grown in the Southeastern Brazil. Borneol (22.4%), *epi*-α-muurolol (13.4%), α-bisabolol (13.1%), precocene I (13.0%), and eucalyptol (7.9%) were the major essential oil constituents. Incubation with essential oil at 200 µg/ml killed all the adult *S. mansoni* worms after 24 h (LC_{50} 117.7 and 103.9 µg/ml at 24 and 120 h of incubation, respectively). At a concentration of 50 µg/ml, the essential oil significantly decreased the motor activity and reduced the percentage of egg development after 120 h. In addition, the essential oil separated all the coupled *S. mansoni* worm pairs into individual male and female at 25 and 50 µg/ml within 120 and 24 h, respectively. This oil was cytotoxic to GM07492-A cells at only concentrations higher than 200 µg/ml (IC_{50} 243.7 µg/ml). These data indicate that LA-EO exhibits moderate *in vitro* activity against adult *S. mansoni* and exerts remarkable effects on eggs development.

Introduction

Schistosomiasis, or bilharzia, is one of the most prevalent parasitosis in the world, second behind malaria. The World Health Organization (WHO) estimates that approximately 200 million people are currently contaminated, and that 800 million are at risk of contracting this disease (Magalhães et al., 2010; Steinmann et al., 2006). Trematode flatworms of the genus *Schistosoma* cause this neglected tropical disease (NTD) (El Shenawy et al., 2008). Praziquantel (PZQ) is currently the most widely employed drug to treat Schistosomiasis, and it plays a key role in population-based disease-control programs (Melo et al., 2011). However, PZQ does not prevent re-infections, is inactive against juvenile schistosomes, and has limited effect

* *Corresponding author.*
E-mail: millercrotti@ffclrp.usp.br (A.E.M. Crotti).

on parasite liver forms (Utzinger et al., 2003). Moreover, the reliance on one single anti-schistosomal drug has culminated in the development of resistant strains schistosome at an alarming rate (Wang et al., 2010; Engels et al., 2002; Ismail et al., 1999).

The search for antiparasitic compounds from natural sources has increased over the last decade, and plants continue to be a major source of biologically active compounds that may provide lead structures to develop new drugs (Magalhães et al., 2010; Pontin et al., 2008). In this scenario, some essential oils can be a promising alternative against *Schistosoma mansoni* (Aguiar et al., 2013; Tonuci et al., 2012; Magalhães et al., 2012; Melo et al., 2011; Caixeta et al., 2011).

Lavandula angustifolia Mill., Lamiaceae, formely *L. officinalis* Chaix or *L. vera* DC, popularly known as lavender, is a widely distributed aromatic and medicinal herb worldwide (Hajhashemi et al., 2003; Cavanagh and Wilkinson, 2002). Its essential oil displays carminative, antiflatulence, and anti-colic properties; aromatherapists use it the holistic relaxant (Hajhashemi et al., 2003). Also, this essential oil acts as a central nervous system depressant, anticonvulsant, sedative, spasmolytic agent, local anesthetic, antioxidant, antibacterial, and mast cell degranulation inhibitor (Cavanagh and Wilkinson, 2002; Kim and Lee, 2002; Lis-Balchin and Hart, 1999; Ghelardini et al., 1999; Hohmann et al., 1999; Tisserand, 1985). Researchers have also investigated the antiparasitic activity of the essential oil from the leaves of *L. angustifolia* against *Giardia duodenalis, Trichomonas vaginalis,* and *Hexamita inflata* (Moon et al., 2006); however, the antischistosomal activity of this oil has not been reported yet.

As part of our ongoing project on the prospection of biologically active natural products (Keles et al., 2011; Peixoto et al., 2011; Ferreira et al., 2010; Vasconcellos et al., 2007), we will report the chemical composition and the *in vitro* schistosomicidal activity of the essential oil of *L. angustifolia* leaves cultivated in Southeastern Brazil against *Schistosoma mansoni* adult worms. Also we will describe the cytotoxic effects of the essential oil of *L. angustifolia* to the normal human fibroblast cell line (GM07492-A).

Materials and methods

Plant material

Lavandula angustifolia Mill., Lamiaceae, was collected at "Sítio 13 de maio" near the city of Franca (20o26'S 47o27'W 977 m, State of São Paulo, Brazil) in May 2010 and identified by Prof. Milton Groppo. A voucher specimen (SPFR 13163) was deposited at the Herbarium of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil (Herbarium SPFR).

Extraction of the essential oil and chemicals

Fresh leaves (250 g) of *L. angustifolia* were submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. After manual collection of the essential oils, anhydrous sodium sulfate was used to remove traces of water, which was followed by filtration. The essential oil samples were stored in an amber bottle and kept in the refrigerator at 4°C until further analysis. The essential oil yield was calculated from the weight of fresh leaves and expressed as the average of triplicate analysis.

GC-FID and GC-MS analysis

The essential oil of *L. angustifolia* (LA-EO) was analyzed by gas chromatography (GC) on a Hewlett-Packard G1530A 6890 gas chromatograph fitted with FID and data-handling processor. An HP-5 (Hewlett-Packard, Palo Alto, CA, USA) fused-silica capillary column (30 m × 0.25 mm i.d.; 0.33 µm film thickness) was employed. The operation conditions were as follows: the column temperature was programmed to rise from 60 to 240°C at 3°C/min and then held at 240°C for 5 min; carrier gas = H_2, at a flow rate of 1.0 ml/min; injection mode; injection volume = 0.1 µl (split ratio of 1:10); injector and detector temperatures = 240 and 280°C, respectively. The components concentrations were obtained by relative peak area normalization (%). The relative areas were the average of triplicate GC-FID analyses.

GC-MS analyses were carried out on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column consisted of Rtx-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary (30-m length × 0.25-mm i.d. × 0.25-µm film thickness). The electron ionization mode at 70 eV was used. Helium (99.999%) was employed as the carrier gas at a constant flow of 1.0 ml/min. The injection volume was 0.1 µl (split ratio of 1:10). The injector and the ion-source temperatures were set at 240 and 280°C, respectively. The oven temperature program was the same as the one used for GC. Mass spectra were taken with a scan interval of 0.5 s, in the mass range from 40 to 600 Da. LA-EO components identification was based on their retention indices on an Rtx-5MS capillary column under the same operating conditions as in the case of GC relative to a homologous series of *n*-alkanes (C_8-C_{24}); structures were computer-matched with the Wiley 7, NIST 08, and FFNSC 1.2 spectra libraries, and their fragmentation patterns were compared with literature data (Adams, 2005). Authentic standards available in our laboratory were also co-eluted with LA-EO to confirm the identity of some essential oil components.

In vitro *studies with Schistosoma mansoni*

The *S. mansoni* LE (Luis Evangelista) strain was maintained in *Biomphalaria glabrat*a snails and Balb/c mice. After eight weeks, *S. mansoni* adult worms (pairs) were recovered under aseptic conditions from mice previously infected with 200 cercariae by perfusion of the livers and mesenteric veins (Smithers and Terry, 1965). The worms were washed in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen), kept at pH 7.5 with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 20 mM, and supplemented with penicillin (100 UI/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum (Gibco). After washing, one pair of adult worms was transferred to each well of a 24-well culture plate containing 2 ml of the same medium and incubated at 37°C in a humid atmosphere containing 5% CO_2 prior to use. The parasites were maintained in RPMI for 24 h, for adaptation to the culture medium. After

this period, LA-EO was dissolved in DMSO (dimethyl sulfoxide) and added to the RPMI medium, to achieve the concentrations of 12.5, 25, 50, 100, and 200 µg/ml. Solutions of the samples were added to the RPMI medium containing four pairs of adult *S. mansoni* worms. The parasites were kept for 120 h and monitored after 24 and 120 h, to evaluate their motor activity and mortality rate. Alterations in the motor activity were classified as either slight or significant. A decrease in the worm movement compared with the negative control for 1 min was considered slight alteration, whereas minimal worm movement was defined as significant alteration (Magalhães et al., 2009). The worms were considered dead when no worm movement was observed for 2 min of examination (Manneck et al., 2010). Also, changes in the pairing, egg production, and egg development were examined by using an inverted microscope (Leitz). To evaluate the egg production, adult worm pairs were cultivated in LA-EO as described before; the number of eggs was counted after 120 h using inverted microscope. To evaluate the eggs development, adult worm pairs were cultivated as described before (in absence of LA-EO) for two days. Next, the worms were removed, and LA-EO was added at same concentrations described before. The period of incubation was five days. Eggs were classified as developed or no developed those previously described in the literature (Michaels and Prata, 1968). RPMI 1640 medium and RPMI 1640 with 0.1% DMSO was used as negative control groups and praziquantel (PZQ) used was the positive control group at a concentration of 12.5 µM (3.9 µg/ml). The experiments were carried out in four replicates and repeated at least three times.

XTT-based cytotoxicity assay

The cytotoxicity was measured using the *in vitro* Toxicology Colorimetric Assay Kit (XTT; Roche Diagnostics) according to the manufacturer's instructions. Human Fibroblasts (GM07492-A – Coriell Cell Repositories) were cultured in HAM-F10 (Sigma-Aldrich) and DMEM ((Dulbecco's Modified Eagle Medium, Sigma-Aldrich) 1:1 culture medium supplemented with 10% fetal bovine serum (Nutricell), antibiotic (10 µg/ml streptomycin and 5 µg/l penicillin; Sigma-Aldrich), and 2.38 mg/ml HEPES [4 - (2-hydroxyethyl)-1-piperazineethanesulfonic acid] (Sigma Aldrich) at 37°C with 5% CO_2. For these experiments, the cells (104 cells/well) were plated onto 96-well microplates, and each well received 100 µl of DMEM containing LA-EO at concentrations ranging from 12.5 to 200 µg/ml, dissolved in 0.2% DMSO. The negative (without treatment), solvent (0.2% DMSO), and positive (25% DMSO) controls were included. After incubation at 37°C for 24 h, the medium was removed; cells were washed twice with 100 µl of PBS and exposed to 100 µl of HAM-F10 medium without phenol red. Next, 15 ml of XTT were added to each well. The microplates were covered and incubated at 37°C for 17 h. Samples absorbance was determined by a multi-plate reader (ELISA, Tecan - SW Magellan vs 5.03 STD 2PC) at a test wavelength of 492 nm and a reference wavelength of 690 nm. Cell viability was expressed as the percentage of untreated cells, which served as the negative control group and was designated as 100%; the results were expressed as a percentage of the negative control (Caixeta et al., 2011). The experiments were performed in triplicate.

Statistical analysis

Results of the antischistosomal and cytotoxic assays were the mean ± SD expressed. The statistical tests were performed with the Graphpad Prism (version 5.0) software. Data were statistically analyzed by one-way analysis of variance, followed by Tukey's multiple comparison test. The LC_{50} (lethal concentration 50%) and the IC_{50} (inhibitory concentration 50%) were calculated from the dose-response inhibition graph.

Results and discussion

Lavandula angustifolia Mill., Lamiaceae, leaves furnished a greenish essential oil (LA-EO) in 0.28% yield (w/w). Table 1 lists the 24 compounds we identified in this oil. GC and GC-MS analysis revealed that monoterpenes (45.0%) are the main LA-EO constituents, being borneol (22.4%), *epi*-α-muurolol (13.4%), α-bisabolol (13.1%), precocene I (13.0%), and eucalyptol (7.9%) its major constituents. Borneol and eucalyptol (1,8-cineole) were also identified as the major constituents in essential oils extracted from the leaves of *L. angustifolia* specimens collected in other countries (Hajhashemi et al., 2003), but the sesquiterpenes *epi*-α-muurolol (13.4%) and α-bisabolol (13.1%), as well as precocene I (13.0%) were not previously reported. In the literature, studies have mainly focused on the essential oils from head flowers of *L. angustifolia*, in which the monoterpenes linalool and linalyl acetate are the major compounds (Machado et al., 2013; Verma et al., 2010; Da Porto et al., 2009; Cavanagh and Wilkinson, 2002). Lavandulol and lavanduloyl acetate are the reportedly characteristic compounds in these essential oils. Thus, our findings corroborate the differences between the chemical compositions of essential oils from the leaves and the flowers of *L. angustifolia*.

Table 2 summarizes the *in vitro* effects of LA-EO against adult *S. mansoni* worms. The positive control (PZQ, 12.5 µM or 3.9 µg/ml) killed parasites within 24 h, without separation of worms, whereas the negative controls (RPMI medium and RPMI medium plus 0.1% DMSO) did not affect mortality, motor activity, or couple separation. Incubation with LA-EO at a concentration of 200 µg/ml culminated in death of all *S. mansoni* adult forms after 24 h. *In vitro*, the LA-EO LC_{50} values against adult worms at 24 and 120 h of incubation were 117.7 and 103.9 µg/ml, respectively. Previous papers showed that male and female *S. mansoni* worms can exhibit different susceptibilities to treatment with some natural products, such as the *Zingiber officinalis* extracts (Sanderson et al., 2002) and the essential oil of *Ageratum conyzoides* (Melo et al., 2011). In this study, male and female *S. mansoni* worms had the same susceptibility to LA-EO. Moreover, 75% of the adult S. mansoni worms incubated with LA-EO at 100 µg/ml displayed minimal motor activities after 24 h of incubation as compared with the negative control (RPMI medium and 0.1% DMSO added to RPMI medium).

It is known that couples of schistosomes can remain paired throughout their lifespan in the blood system of their vertebrate host, culminating in intense oviposition rate. This results in immunopathological lesions characterized by inflammation

Table 1
Chemical composition of the essential oil of *L. angustifolia* leaves.

Compound	RT (min)	RI_{exp}	RI_{lit}	RA %	Identification
α-pinene	5.94	934	939	0.7	RL, MS, Co
camphene	6.34	949	953	0.5	RL, MS
β-pinene	7.11	978	980	1.1	RL, MS, Co
myrcene	7.44	991	991	0.7	RL, MS
3-carene	8.09	1012	1011	0.9	RL, MS
β-phellandrene	8.70	1030	1031	3.4	RL, MS
eucaliptol	8.77	1032	1033	7.9	RL, MS, Co
camphor	12.88	1146	1143	3.5	RL, MS, Co
borneol	13.70	1167	1165	22.4	RL, MS, Co
4-terpineol	14.13	1179	1179	0.9	RL, MS
cryptone	14.50	1188	1188	1.0	RL, MS
α-terpineol	14.65	1192	1189	1.2	RL, MS
myrtenol	14.90	1199	1186	0.4	RL, MS
unknown	16.14	1230		0.5	RL, MS
carvone	16.76	1245	1243	0.4	RL, MS
eugenol	21.24	1359	1356	2.0	RL, MS, Co
β-cariophyllene	23.63	1421	1418	3.2	RL, MS, Co
precocene I	25.26	1464	1461	13.0	RL, MS, Co
β-bisabolene	26.97	1510	1509	0.8	RL, MS
γ-cadinene	27.17	1515	1513	2.9	RL, MS
caryophyllene oxide	29.67	1584	1581	4.5	RL, MS, Co
carotol	30.80	1617	1614	0.7	RL, MS
epi-α-uuurolol	31.71	1643	1643	13.4	RL, MS
α-bisabolol oxide	32.17	1656	1655	0.9	RL, MS
α-bisabolol	33.18	1685	1683	13.1	RL, MS, Co
Total				100%	

Monoterpene hydrocarbons, 7.3%; Oxygenated monoterpenes, 37.7%; Sesquiterpene hydrocarbons, 6.9%; Oxygenated sesquiterpenes, 32.6%; Others: 15%; Not identified, 0.5% ; RT, retention time in an Rtx-5MS column; RI, retention indices relative to n-alkanes C8-C20 on the Rtx-5MS capillary column; RA, relative area (peak area relative to the total peak area in the GC-FID chromatogram), average of three replicates; RL, comparison of the retention index with the literature; MS, comparison with the mass spectrum reported in the literature (Adams, 2005); Co, co-elution with authentic standards available in our laboratory.

and fibrosis in the target organs (Knobloch et al., 2006). To investigate how LA-EO affects how these important features associated with the schistosomicidal activity, we conducted additional experiments to assess the impact of this oil on the worms pairing. Table 2 shows that LA-EO at concentrations of 25 and 50 µg/ml separated 100% of the coupled pairs of worms after 120 and 24 h of incubation, respectively. The parasites belonging to the negative and solvent control groups (RPMI 1640 medium and DMSO 0.1% plus RPMI 1640 medium) remained coupled, even after 120 h.

Some plant-derived essential oils, such as those obtained from *Ageratum conyzoides* (Melo et al., 2011), *Plectranthus neochilus* (Caixeta et al., 2011), *Tagetes erecta* (Tonuci et al., 2012), and *Bidens sulphurea* (Aguiar et al., 2013), affect eggs production. At concentrations higher than 50 µg/ml, these essential oils significantly reduced the number of eggs when compared to the negative control groups at 120 h. However, the effects of these essential oils on the eggs production could stem from the separation of the couple pairs of worms, which were also observed concentrations at these. Considering this separation that could prevent any reproductive process, the effects of these essential oils on the eggs production were evaluated at a concentration of 10 µg/ml, in which no separation of the couple pairs of worms was observed. At this concentration, the number of eggs in the groups incubated with the essential oil of *B. sulphurea* was reduced to 70% as compared to the negative control groups after 120 h (Aguiar et al., 2013), whereas the essential oils of *A. conyzoides*, *P. neochilus* and *T. erecta* and had no effects on the eggs production. In this study, we observed that LA-EO decreased slightly the number of eggs as compared with the negative control, at all the tested concentrations (date not shown). However, this effect may stem from the separation of the coupled pairs of worms, which occurred at all the tested concentrations and prevented any reproductive process. On the other hand, we observed that LA-EO significantly diminished the percentage of developed eggs as compared with the positive control at concentrations higher than 25 µg/ml in a dose-response dependent manner at 120 h of incubation (Fig. 1).

Finally, we assessed the cytotoxicity of LA-EO on human fibroblasts GM07492-A exposed to LA-EO concentrations of 12.5, 25.0, 50.0, 100.0, 200.0, and 400.0 µg/ml for 24 h using an XTT cell viability assay kit. We determined the viability of the cultures by establishing a relation between the absorbance values obtained for the treated and untreated (control) groups, those shown in Fig. 2. We verified significant cytotoxicity only after exposure to LA-EO concentrations higher than 200 µg/ml for 24 h (IC_{50} = 243.7 µg/ml). These concentrations were higher than those for which the LA-EO displayed *in vitro* activity schistosomicidal , that suggesting this oil is not cytotoxic at the active concentrations (LC_{50} = 117.7 and 103.9 µg/ml at 24 and 120 h, respectively).

Terpenes, especially monoterpenes and sesquiterpenes, account for the hydrophobic nature of essential oils (Burt et al., 2005). This hydrophobicity allows these compounds to freely permeate the cell membranes, killing the parasites by affecting their metabolic pathways or organelles (Knobloch et al., 1989). On the other hand, essential oils themselves could also interact with the parasite cell membrane and cause drastic physiologic

Table 2
In vitro effects of the essential oil of *Lavandula angustifolia* (LA-EO) against adult *S. mansoni* worms.

Group	Incubation period (h)	Separated couples (%)	Dead worms (%)		Motor activity			
					Slight (%)[d]		Significant (%)[d]	
			M	F	M	F	M	F
Control[a]	24	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0
DMSO 0.1%[b]	24	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0
PZQ[c]	24	0	100	100	0	0	0	0
	120	0	100	100	0	0	0	0
12.5 µg/ml	24	0	0	0	100	100	0	0
	120	25	0	0	100	100	0	0
25 µg/ml	24	0	0	0	100	100	0	0
	120	100	0	0	100	100	0	0
50 µg/ml	24	100	0	0	100	100	0	0
	120	100	25	25	0	0	75	75
100 µg/ml	24	25	25	25	0	0	75	75
	120	25	25	25	0	0	75	75
200 µg/ml	24	25	100	100	0	0	0	0
	120	25	100	100	0	0	0	0

M, males; F, females.
[a]RPMI 1640.
[b]0.1% DMSO + RPMI medium.
[c]PZQ: Praziquantel.
[d]Slight was defined as a reduction in movement compared with the negative control. Significant was defined as minimal movement observed for 1 min. Dead worm was defined when no movement was observed for at least 2 min.

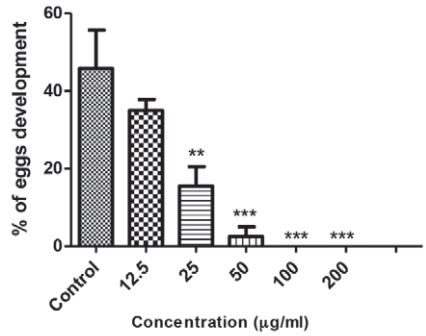

Figure 1 - *In vitro* effects of LA-EO on eggs development. Quantitative analysis of phenotype development. After treatment, the eggs were examined microscopically and scored as developed or undeveloped on the basis of the presence or absence of the miracidium. Data presented are the mean of the eggs developed from three separate experiments. Control, eggs in 0.1% DMSO.
$^{**}p < 0.01$.
$^{***}p < 0.001$.

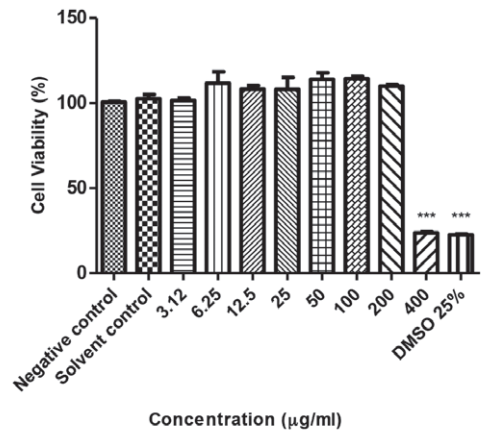

Figure 2 - Effects of LA-EO on the viability of GM07492-A cells.
$^{*}p < 0.001$.

changes, resulting in loss of membrane permeability and consequent cell death (Bakkali et al., 2008; Knobloch et al., 1989). However, considering the number of constituents and the synergic and antagonic interactions between them, it is also possible that the essential oils have other cell targets than cell membranes, such as lipids and proteins (Borges et al., 2012; Bakkali et al., 2008).

The monoterpenes borneol and eucalyptol, the LA-EO major chemical constituents, have been identified also as the main compounds in the many essential oils exhibiting anti-parasitic activity. They are the major constituents of the EOs of *Thymus capitelatus*, which displayed leishmanicidal activity against the promatigote forms of *Leishmania infantum* (Machado et al., 2010), and the EO of *Curcuma longa*, which displayed moluscidal activity against adult *Biomphalaria glabrata*, the latter being the intermediate host of *Schistosoma mansoni* (da Silva Filho et al., 2009). Also, borneol has been associated with the anti-helmintic effects of many medicinal plants (Jaiswal et al., 2011). Eucaliptol was reported as the major compound in the essential oils of *Piper cubeba* (Magalhães et al., 2012) and *Eucalyptus camaldulensis* (Habila et al., 2010), which were active against *S. mansoni*, and *Trypanosoma brucei* and *T. evansi*, respectively. Precocene I, one of the major LA-EO constituents, was reported to be inactive against *S. mansoni* (Melo et al., 2011). However, these compounds may be involved in intricate synergic and/or addictive effects between these compounds and other minor LA-EO constituents (Melo et al., 2011).

Regarding to the cytotoxicity, the essential oil of *L. angustifolia*, consisting mainly of linalyl acetate (51%) and linalool (35%), was reported to be cytotoxic to skin cells *in vitro* (endothelial cells and fibroblasts) at a concentration of 0.25% (v/v) in the case of HMEC-1, HNDF, and 153BR cell types (Verma et al., 2010). Considering that linalyl acetate and linalool were previously described as the cytotoxic compounds of *L. angustifolia* essential oil, the low cytotoxicity of LA-EO on GM07492-A human fibroblasts may be due to the absence of linalool and linalyl acetate.

The absence of cytotoxicity reported in human fibroblasts is worth of interest considering that schistosomiasis affects millions of people worldwide. According to our results, the cytotoxic effects of LA-EO were expressed in human fibroblasts at a concentration of 400 µg/ml. On the other hand, treatment with LA-EO at 200 µg/ml for 24 induced parasite death. In this sense, we reported results from two different experimental models. Despite the ability of hydrophobic compounds present in LA-EO to cross the cell membranes, it is supposed that different cell response are operating, especially when we consider cells from different species. *S. mansoni* tegument exhibits important function related to protection against host immune system and absorption of nutrients and other molecules related, being of essential importance for parasite adaptation and survival. Moreover, Pereira et al. (2014) reported that 365 µg/ml of tea tree oil from *Melaleuca alternifolia* did not induce cytotoxicity neither genotoxicity in human lymphocytes treated for 48 h (Pereira et al., 2014). This essential oil was predominantly constituted by terpene-type components (98%). Therefore, in the present study we suppose that the parasite cell death is related to thedirect effect of LA-EO on the *S. mansoni* tegument.

In summary, the essential oil of *L. angustifolia* exhibits moderate activity against adult *S. mansoni* worms *in vitro*, killing parasites and prompting interesting effects on the separation of the coupled pairs of worms and their motor activities. This oil also affects eggs development in a dose-dependent manner. These date indicate that LA-EO exhibits moderate *in vitro* activity against adult *S. mansoni* and exerts remarkable effects on egg development, besides displaying low cytotoxicity.

Authors' contributions

MG contributed by collecting and identifying the plant sample and establishing the herbarium. ALLM contributed by running the laboratory work, analyzing the data, and drafting the paper. GPGV and RAS contributed with the biological studies. LGM, VR, and WRC supervised the biological assays and critically read the manuscript. AEMC designed the study, supervised the laboratory work, and critically read the manuscript. All the authors have read the final manuscript and approved the submission.

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