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Phytotherapeutic potential of Lamiaceae essential oils and their monoterpenes against *Giardia duodenalis*.

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ABSTRACT

Lamiaceae and Asteraceae plant species have been widely used in Mediterranean ethnomedicine for gastrointestinal disorders. They are also known for their antioxidant, anti-inflammatory, anti-bacterial, anti-parasite, and anti-virus properties. *Giardia duodenalis* is the most prevalent intestinal protozoon in children and young dogs worldwide. Its zoonotic potential and frequent therapeutic failures with nitroimidazoles underscore the urgent need for alternative treatments. This study investigated the antigiardial activity of essential oils (EOs) from 22 medicinal plants belonging to Lamiaceae and Asteraceae, together with their major constituents. EO composition was determined by a metabolomic approach (GC-MS). Parasite metabolic activity was assessed using the MTT assay, and ultrastructural changes were examined by Transmission Electron Microscopy. The strongest antigiardial effects were observed with *Lavandula luisieri*, *Thymus vulgaris, Mentha suaveolens, Satureja montana* (IC_{50} <25), *L. lanata*, and *T. zygis,* (IC_{50}= 27.9–71.5 μg/ml). The highest selective indexes were obtained with γ-terpinene, caryophyllene oxide, carvacrol and thymol (SI≥1.3–2.4). Synergistic interactions were detected with linalyl acetate and linalool (present in *Lavandula* EOs), linalyl acetate with ρ-cymene or thymol, or combinations of ρ-cymene, γ-terpinene, thymol, and carvacrol (present in *Satureja* EOs). Transmission Electron Microscopy revealed membranolysis, enlarged periplasmic vacuoles, and cytoplasmic loss in trophozoites exposed to γ-terpinene after 1 h. These findings provide phytotherapeutic evidence supporting essential oils from *Lavandula*, *Mentha*, *Thymus,* and *Satureja* as promising antigiardial agents. Their main components γ-terpinene, caryophyllene oxide, carvacrol and thymol could have potential applications in veterinary parasitology.

1. Introduction

Giardia duodenalis (*G. duodenalis*) is a common parasite of mammals, including dogs, cats, and farm animals, among others. While genotype A and B have been described in many species, including humans, other genotypes seem more specific (Ryan and Caccio, 2013). During the first year of life, dogs prevalences range from 17 % in the general dog population up to 46 % in shelters or other facilities, and it is associated in

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most cases with clinical signs (Ryan and Caccio, 2013; Drake et al., 2022, Mateo et al., 2023). It is also the most common parasite in the gastrointestinal tract of humans, with world prevalences ranging from 0.4 % to 7.5 % in developed countries and 8–30 % depending in developing countries (Feng and Xiao, 2011; European Centre for Disease Prevention, 2022). It is particularly prevalent in children between 0 and 2 years of age, ranging from 37.7 % to 96.4 % in some countries, depending on the geographical location, socioeconomic status, and recent metronidazole treatment, and more than 40 % of children displaying repeated infections (Rogawski et al., 2017).

Nitroimidazoles remain the cornerstone of giardiasis therapy; however, over the past decades, an increasing number of cases of reduced therapeutic efficacy and refractory infections—defined as giardiasis unresponsive to standard treatment—have been documented in both canine and human populations. Most of the refractory cases are considered as resistant to the employed treatment but others are related to deficient immune responses (Nabarro et al., 2015; Carter et al., 2018; Ciuca et al., 2021). This issue has gained particular relevance among travelers, with treatment resistance reported in up to 50 % of confirmed cases, especially among individuals returning from Asia (Nabarro et al., 2015). Furthermore, substantial evidence indicates cross-resistance across structurally related agents, notably the 5-nitroimidazoles (Upcroft and Upcroft, 2001). Moreover, in certain cases, an additional complication arises in the form of inflammatory responses, attributable to metronidazole-induced dysbiosis (Pilla et al., 2020). Besides, in recent years, EU regulation 2019/6 focuses on the antibiotic resistances, complicating the veterinary prescription of antibiotics (Regulation, 2019). With this scenario, it is essential to find alternatives or complementary treatments for giardiasis.

Natural products are composed of secondary metabolites that help living organisms to defend against pathogens and environmental stress. Products extracted from plants have been employed successfully for centuries against disease, and many of the components displayed anti-inflammatory, antioxidant, anti-bacteria, anti-virus and anti-parasite properties (Aziz et al., 2018; Ramsey et al., 2020). In traditional medicine, Lamiaceae and Asteraceae products are chiefly applied to gastrointestinal disorders, particularly in Mediterranean countries such as Morocco and Pakistan (Jamila and Mostafa, 2014; Rahman et al., 2016; Es-Safi et al., 2020; Redouan et al., 2022). Among these disorders, diarrhea and virus, bacteria and parasite infections are frequently mentioned.

Plant-derived antigiardial (AG) agents represent promising alternative and complementary therapeutic resources (Vidal et al., 2007; Alnomasy et al., 2021). Lamiaceae and Asteraceae were most frequently investigated (30 % and 13.5 %, respectively), whereas Apiaceae, Myrtaceae, Amaryllidaceae, Cucurbitaceae, and Zingiberaceae were less commonly assessed (4.5–10.5 % of studies). In the search of AG agents, Lamiaceae are also interesting since it comprises plants that are active against more than one parasite, such as *Melissa officinalis*, *Origanum vulgare*, or *Thymus vulgaris* (Anthony et al., 2005). Most of the studies performed with plants employed aerial parts, including leaves, but seeds, fruits, grain, stems, peel, flowers, bulbs and other minor components have been explored. Among the extracts, aqueous extract and essential oils (EOs) predominate in the studies (30 % and 25.4 % of the studies), although there are other extraction methods, such as ethanolic, methanolic, hydroalcoholic, chloroform, petroleum-ether, and hexane extracts (Alnomasy et al., 2021).

Some of the most active natural products against *Giardia* trophozoites and barely cytotoxic were extracted from plants of the Lamiaceae family, such as *Mentha x piperita*, *Ocimum basilicum*, *Salvia mirzayanii, Thymus capitata Origanum virens*, and *Thymus zygis* subsp. *sylvestris*, (Vidal et al., 2007; El-Badry et al., 2010; Machado et al. 2010a,2010b; Shaverdi et al., 2024). Extracts from Asteraceae have demonstrated good AG activity, and among them, we can highlight *Ageratum conyzoides* (Pintong et al., 2020).

Natural products from other plants, such as the EO from the Myrtaceae *Syzygium aromaticum*, also displayed an interesting activity against *Giardia* trophozoites (Machado et al., 2011). Garlic (*Allium sativum*) extracts are less effective, although some compounds obtained from garlic were highly effective against *Giardia* trophozoites (Argüello-García et al., 2018; Harris et al., 2000).

Excluding compounds with cytotoxic effects or without cytotoxicity assays, only a few pointed out as alternatives to fight *Giardia*, considering compounds with $IC_{50} \leq 100$ μg/ml. Linearolactone, a diterpenoid compound isolated from *Salvia* species from Mexico (Calzada et al., 2015), and eugenol, a major component of *Syzygium aromaticum* EO (Machado et al., 2011).

To increase the number of active natural products or compounds against *Giardia* spp., we analyzed the AG activity of 30 EOs from 22 Lamiaceae and Asteraceae medicinal plants obtained by two extraction methods, hydrodistillation (HD) and steam distillation (SD), to explore their utility as alternative or complementary treatments against giardiasis. We also tested cytotoxicity and AG activity of their main components alone or in combinations to investigate possible synergies. Finally, we explored the morphological alterations produced by the compound with the lowest IC_{50} value.

2. Methods and material

2.1. Plants and essential oils extraction

Essential Oils were obtained from plants belonging to the families Lamiaceae and Asteraceae. In total, 30 EOs from 22 different plants were used in the present study. All plants were subjected to HD to obtain 22 EOs. Besides, 8 plants were subjected to SD to obtain 8 EOs (Table 1) (*Lavandula* x "*intermedia*" varieties "Abrial" and "Super", *Lavandula luisieri*, *Lavandula angustifolia, Lavandula angustifolia var. mallete, Mentha suaveolens*, *Origanum virens*, *Satureja montana*, *Thymus vulgaris*, *Thymus zygis*).

All the plant species have been domesticated or are in culture. Seeds from the rest of the plants were obtained at the germplams banks of CITA (Centro de Investigación y Tecnología Agroalimentaria de Aragón, Plant Science Department, Zaragoza, Spain). The plant species were identified by Dr. Daniel Gómez (Instituto Pirenáico de Ecología, IPE-CSIC). Location, identification, and voucher numbers of the remaining species have been previously reported (Bailén et al., 2022). The plant names have been checked with http://www.theplantlist.org.

Table 1
Families, plant species and extraction methods of the EOs employed in the present study.

Family	Genus	Plant species	EO extraction method
Asteraceae	*Ditrichia*	*D. graveolens*	HD
	Santolina	*S. chamaecyparissus*	HD
	Tanacetum	*T. vulgare*	HD
Lamiaceae	*Lavandula*	*L. angustifolia*	HD
		L. intermedia "Abrial"	HD, SD
		L. intermedia "Grosso"	HD
		L. intermedia "Super"	HD, SD
		L. lanata	HD
		L. luisieri	HD, SD
		L. angustifolia var. mallete	HD
	Mentha	*M. suaveolens*	HD, SD
	Origanum	*O. majorana*	HD
		O. virens	HD, SD
	Rosmarinus	*R. officinalis*	HD
	Salvia	*S. officinalis*	HD
		S. blancoana	HD
		S. hybrid	HD
		S. sclarea	HD
	Satureja	*S. montana*	HD, SD
	Thymus	*T. mastichina*	HD
		T. vulgaris	HD, SD
		T. zygis	HD, SD

The aerial part of each plant was maintained during 7 days at room temperature and protected from light. The hydrodistillation process was conducted in triplicate, employing 100 g of dried aerial part and 2 L of water in a Clevenger-type apparatus, according to the procedure recommended by the European Pharmacopeia (European Pharmacopoeia, 2025). The oils were dried, filtered, and stored at 4ºC until use and determination of the EOs composition. The steam distillation procedure was performed using 60 kg of fresh biomass harvested at the flowering stage. A stainless-steel pilot extraction plant with a reducing pressure valve was used, with an operating pressure of 0.45 bar. The aqueous phase (hydrolate) was decanted from the EOs collected in the condensation section and filtered, as previously described (Julio et al., 2017). The obtained EOs were stored at 4ºC for a maximum of two years until all replicas were performed.

2.2. Identification of EOs main components

The main compounds of the EOs from the plants were identified by a metabolomic approach, using gas chromatography- mass spectrometry (GC-MS) employing a Shimadzu GC2010-Plus coupled to a Shimadzu GCMS-QP2010-Ultra mass detector with a single Quadrupole analyser. The electron impact ionization source was at 70 eV, employing Helium as gas carrier. The conditions have been described elsewhere (Bailén et al., 2022). Briefly, samples were dissolved in 100 % dicholoromethane (4 μg/μl) before injected. Two technical replicates were conducted, and no differences were observed.

Chromatography was carried out using a Teknokroma TRB-5 (95 %) Dimethyl- (5 %) diphenylpolysiloxane capillary column, 30 m x 0.25 mm ID and 0.25 μm phase thickness. The initial column temperature was 70∘C, heating up to 290∘C at 6∘C /min and leaving at 290∘C for 15 min. The split mode injection used 1 μl of sample with a split ratio (20:1) employing a Shimadzu AOC-20i automatic injector. The injector temperature was 300∘C, the transfer line temperature connected to the mass spectrometer was 250∘C, and the ionization source temperature was 220∘C.

The mass spectra, retention time, and retention indexes were compared with data of the Wiley database (Wiley 275 Mass Spectra Database, 2001) and NIST 17 (NIST/EPA/NIH Mass Spectral Library). Quantification was conducted considering the relative area percentages of all peaks from the chromatograms. Trans-α-necrodyl acetate from *L. luisieri* was identified from a compound previously isolated and it is no further available.

2.3. Pure compounds

After determining the composition of the analyzed EOs, compounds that were available and representing at least more than 5 % of the EOs with lower IC_{50} values, were employed in AG activity assays. Compounds not identified, not commercially available or not easy to isolate or to obtain were excluded (including piperitenone, piperitenone oxide, and trans-α-necrodyl acetate).

Monoterpenes and sesquiterpenes were obtained from commercial sources, except for camphor that was isolated in the facilities of ICA-CSIC laboratory. Linalool, limonene, thymol, α-pinene, α-terpineol, β-pinene, camphene, β- caryophyllene, and caryophyllene oxide, as well as metronidazole were obtained from Sigma Aldrich; linalyl acetate, γ-terpinene, and ρ-cymene from Acros Organics; 4-terpineol from Merck Life Sciences; α and β thujone from Phytolab; borneol, carvacrol,1.8-cineole, D-fenchone, and ocimene from Fluka. All the companies were in Madrid, Spain.

2.4. Giardia duodenalis culture

Strain WB from *G. duodenalis* (obtained from a duodenal aspirate of a human) was purchased at the American Type Culture Collection (ATCC, isolate #30957™). The trophozoites were grown in Keysterś modified TYI-S-33 medium at 37ºC in anaerobiosis. Trophozoites were grown in 15 ml polypropylene tubes, and the medium was refreshed every 2–3 days. When a monolayer was observed, the tube was cooled down at 4º C during 30–40 min for detachment and split in half. Trophozoites were preserved in 2 ml cryovials containing 5 % dimethyl sulfoxide (DMSO) in culture medium at −80ºC for medium term storage, or in liquid nitrogen for long-term storage.

2.5. Anti- G. duodenalis activity assays of EOs and pure compounds

The metabolic activity of the trophozoites was determined employing the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method (Bénéré et al., 2007) with modifications (Arroyo Díaz et al., 2023). Briefly, 145 μl of a solution containing 1×10^6 trophozoites/ml in TYI-S-33 medium were seeded in 96 well microtiter plates (Falcon BD, Fischer scientific, Madrid, Spain), and 5 μl of each EO or pure compound solution in DMSO (≤1 %) were added in each well at different concentrations, in quadruplicate, and the plates were immediately sealed. A minimum of three replicas were performed for each EO/pure compound. EOs were evaluated at 800, 400, and 200 μg/ml, while pure compounds were tested at 100, 10 or 1 μg/ml. In case of significant AG activity of the EOs at 200 μg/ml, concentrations of 100, 50 and 25 μg/ml were additionally evaluated. In the case of pure compounds with IC_{50} values lower than 100 μg/ml, at least four intermediate concentrations between 100 and 1 μg/ml were also assayed to determine the inhibitory concentration of 50 % (IC_{50}) dose. Assays were incubated at 37ºC during 24 h, and then TYI-S-33 media was aspirated from each well and replaced for 100 μl of MTT-PMS (phenazine methosulfate, SIGMA, Madrid, Spain) solution (0.25 mg/ml MTT + 0.06 mg/ml PMS in PBS). After 75 min. at 37ºC, 100 μl of sodium dodecyl sulphate were added to each well and incubated for 30 more minutes. After that, the plates were read in a spectrophotometer at 570 nm (Biotek 800ST microplate reader).

The AG activity was calculated as a percentage of the metabolic activity of the trophozoites, employing the formula: %AG= 100-[(As-Ab)/(Ac-Ab)]x100, where As is the absorbance of the sample (tested EO or compound), Ab is the absorbance of the blank (only culture medium), and Ac is the absorbance of the control (trophozoites culture without treatment and with 1 % DMSO). Metronidazol (Sigma Aldrich, Madrid, Spain) was employing as the reference drug for *Giardia*.

Those EOs with IC_{50} values lower than 200 μg/ml, according to previous criteria of promising AG activity (Machado et al., 2010b; Calzada et al., 2015), were subjected to further analysis, while EOs with IC_{50} values higher than 200 μg/ml were discarded and they were not employed in more experiments.

2.6. Determination of synergisms in compounds combination

The synergistic activity of pure compounds was determined employing combinations of pure compounds from the EOs with low IC_{50} values, following the method previously described, and employing a final concentration of 100 μg/ml (both compounds). The following combinations of paired products were tested at 1:1 (50 μg/ml +50 μg/ml), 2:1 (66.6 μg/ml + 33.3 μg/ml) and 1:2 (33.3 μg/ml + 66.6 μg/ml) proportions: linalyl acetate + 1,8-cineol; linalyl acetate + camphor; linalyl acetate + γ-terpinene; linalyl acetate + α-terpineol; linalyl acetate + ρ-cymene; linalyl acetate + thymol; linalyl acetate + linalool; linalool + 1,8-cineol; linalool + camphor; linalool + α-terpineol; thymol + γ-terpinene; thymol + ρ-cymene; thymol + carvacrol; ρ-cymene + γ-terpinene; ρ-cymene +carvacrol; γ-terpinene + carvacrol; camphor + 1,8-cineol. Non-available pure compounds, compounds with IC_{50} higher than 100 μg/ml or compounds with cytotoxic activity were excluded from the synergism study.

Synergism was considered to occur when the AG activity of the compound pair (observed AG activity) was statistically higher than the sum of the AG activities of each compound tested individually (expected

AG activity) (Guardo et al., 2017).

2.7. Cytotoxicity assays and selectivity index

Cytotoxicity assays were conducted in 96 wells plates following the MTT method and employing Vero cells (a cell line derived from the kidney of African green monkey) (González-Coloma et al., 2002). Briefly, cells were cultured in 10 % foetal calf serum (FCS, Sigma, Madrid, Spain) Dulbecco's modified Eagle's minimal essential medium (DMEM, Avantor, Llinars del Vallès, Barcelona) at 37ºC and 5 % CO_2 in humidified atmosphere. Serial dilutions of the pure compounds were added to each well containing 1×10^4 cells/well in quadruplicate. To determine the concentration that kill 50 % of the cells, cytotoxicity concentration 50 (CC_{50}), the compounds were employed at final concentrations of 100, 75, 50, 25, 10 and 1 μg/ml.

The minimum selectivity index (SI) of the compounds with IC_{50} values lower than 100 μg/ml was calculated following the formula $SI=CC_{50}/IC_{50}$, considering that the highest CC_{50} assayed was 100 μg/ml. Those products with SI higher than 1 were considered as potential AG compounds. Also, α-pinene could be considered as a potential AG compound, since the cytotoxicity was close to our maximum concentration tested, and the SI was 3.5. The therapeutic window will be broader with precise cytotoxicity value of each compound, since the best candidates demonstrated higher CC_{50} values in our conditions.

2.8. Transmission electron microscopy

Giardia trophozoites were cultured as previously described and treated with γ-terpinene (100 μg/ml). Two tubes were used per time point, and the experiment was repeated twice. Then, parasites were processed for Transmission Electron Microscopy (TEM) analysis to look for ultra morphological alterations. Trophozoites treated with the compound, as well as control trophozoites without treatment, were processed after 60 min and 24 h exposition.

Giardia cultures were cooled down at 4ºC for 40 min to detach the trophozoites. The tubes were centrifuged at 800 x *g* for 5 min and washed with sterile cold PBS twice. The pellets containing the trophozoites were transferred to Eppendorf tubes for the fixation process. The concentrated parasites were fixed for 4 h at 4ºC in Karnovsky buffer (4 % paraformaldehyde, 2.5 % glutaraldehyde (Fisher scientific, Madrid, Spain) in Milloning phosphate buffer (Millonig, 1961). Then, trophozoites were washed four times with Milloning buffer, and left overnight at 4ºC in 1 ml of the last washing buffer. After centrifugation, the trophozoites were post-fixed in 1 % osmium tetroxide and potassium ferrocyanide 1.5 % for 1 h, followed by three washing in Milli Q water. A dehydration procedure was conducted in 15 min steps of acetone in increasing concentrations. The acetone was substituted by SPURR resin (Iberlabo S.A., Madrid, Spain) by washings in growing concentrations of resin: acetone 1:3 for 1 h, 1:1 for 1 h, 3:1 for 2 h, and then pure resin overnight. The samples were embedded in new resin and were polymerized at 65ºC for 48–72 h. The embedded samples were sent to the Spanish National Centre for Electron Microscopy, where ultrathin sections (60 nm depth) where cut, loaded in a copper grid and examined in a JEOL JEM-1400 Electron Microscope operating at 80 kV.

2.9. Statistical analysis

The data obtained was analyzed employing STATGRAPHICS Centurion 19. A linear regression analysis was employed to determine IC_{50} anti-*Giardia* activity, as well as CC_{50} cytotoxic activity (percentage of cell viability on log-dose).

The viability of cells and trophozoites was assessed for each compound through a dose-response experiment to determine their relative potency, expressed as CC_{50} or IC_{50} values. The IC_{50} (μg/ml) represents the concentration of EOs or pure compounds required to achieve 50 % mortality of trophozoites, whereas the CC_{50} (μg/ml) indicates the concentration needed to induce 50 % mortality in Vero cells.

The minimum selectivity index (SI) was calculated for pure compounds using the formula $SI = CC_{50}/IC_{50}$. Compounds with an SI greater than one were considered promising AG agents, as they exhibit higher toxicity toward protozoan than mammalian cells.

Normality of the variables "expected" and "observed" antigiardial activity (synergies of pure compounds) was assessed using the Shapiro–Wilk test. Since the data were normally distributed, the unpaired Student's *t*-test was applied.

3. Results

3.1. Anti-Giardia activity of EOs

The AG activity of the different EOs varies greatly between several plant species. All the EOs analyzed were active (80 % AG activity) against *Giardia*, at least at 800 μg/ml (Tables 2 and 3). The IC_{50} values obtained from the essential oils of three plants were comparable, regardless of whether they were extracted using HD or SD (*M. suaveolens*, *T. vulgaris* and *T. zygis*). Three of these EOs displayed better IC_{50} values when extracted by HD (*L. "Super"*, *L. "Abrial"* and *O. virens*), while only two plants displayed slightly better results with EOs extracted by SD (*L. luisieri*, *S. montana*).

Considering the IC_{50} values, 15 EOs were selected for further analysis, since their IC_{50} values were lower than 200 μg/ml. They belonged to the genera *Lavandula*, *Thymus*, *Mentha*, *Satureja*, *Origanum* and *Salvia* (Table 2). The best IC_{50} values were obtained with *T. vulgaris* and *M. suaveolens* EOs obtained with both extraction methods (IC_{50} value < 25 μg/ml), and *S. montana* and *L. luisieri* obtained with SD extraction method (IC_{50} value < 25 μg/ml). EOs from *T. zygis*, *Lavandula x intermedia "Super"* and *"Abrial"*, *S. sclarea*, and *O. virens* displayed slightly higher IC_{50} values (66.9 – 199.4 μg/ml).

The EOs with an IC_{50} higher than 200 μg/ml (n = 15) were not subjected to further analysis (Table 3), except for the determination of their composition. IC_{50} values ranged from 209.6 to 555.5 μg/ml. These EOs were extracted from the following plants and methods of extraction: *L. angustifolia* HD, *L. intermedia* "Abrial" SD, *L. intermedia* "Grosso" HD, *L. intermedia* "Super" SD, *L. angustifolia var. mallete* HD, *D. graveolens* HD, *O. virens* SD, *O. majorana* HD, *R. officinalis* HD, *S. hybrid* HD, *S. officinalis* HD, *S. blancoana* HD, *S. chamaecyparissus* HD, *T. mastichina* HD, and *T. vulgare* SD.

Table 2
Anti-*Giardia* activity of EOs from different plant species and extraction methods evaluated with IC_{50} values lower than 200 μg/ml (ordered from lower to higher IC_{50}).

Plant species	EO extraction method	IC_{50} in μg/ml (95 % Confidence interval)
L. luisieri	SD	< 25
T. vulgaris	SD	< 25
T. vulgaris	HD	< 25
M. suaveolens	SD	< 25
M. suaveolens	HD	< 25
S. montana	SD	< 25
L. lanata	HD	27.9 (25.6–30.4)
L. luisieri	HD	32.1 (24.2–42.7)
T. zygis	HD	66.9 (65.1–68.7)
T. zygis	SD	71.5 (62.4–81.9)
S. sclarea	HD	88.2 (68.9–112.9)
S. montana	HD	109.2 (100.9–118.2)
Lavandula x intermedia "Super"	HD	111.3 (100.7–123.0)
Lavandula x intermedia "Abrial"	HD	113.0 (100.9–126.4)
O. virens	HD	199.4 (185.0–214.9)

Table 3
Anti-*Giardia* activity of EOs from different plant species and extraction methods evaluated with IC_{50} values higher than 200 μg/ml.

Plant species	EO extraction method	IC_{50} in μg/ml (95 % Confidence interval)
S. chamaecyparissus	HD	209.6 (203.0–216.5)
L. intermedia "Super"	SD	251.8 (192.3–329.5)
L. x intermedia "Abrial"	SD	271.5 (260.1–283.3)
R. officinalis	HD	272.4 (245.9–301.7)
L. x intermedia "Grosso"	HD	274.8 (255.4–295.6)
O. virens	SD	275.9 (266.2–285.9)
S. officinalis	HD	281.9 (275.5–288.5)
D. graveolens	HD	284.2 (281.3–287.2)
O. majorana	HD	288.9 (280.8–297.2)
T. mastichina	HD	290.4 (279.5–301.7)
T. vulgare	SD	293.5 (279.9–307.9)
L. angustifolia var. mallete	HD	364.1 (342.4–387.2)
S. blancoana	HD	390.1 (367.6–413.9)
L. angustifolia	HD	480.9 (426.7–541.8)
S. hybrid	HD	555.5 (533.9–577.8)

3.2. Main components of the EOs with low IC_{50} values

The main components of the different EOs will be described from higher to lower activity against the parasite, and in active EOs (>80 % AG activity) (Table 4, supplementary table 1). In general, the main components of the tested EOs were similar with both extraction methods, when employed.

Piperitenone (20.8–53.4 %) and piperitenone oxide (22.8–35 %) were the most abundant components of *M. suaveolens* EOs, which displayed IC_{50} values lower than 25 μg/ml. At minor proportions, limonene (5.6–6.6 %), germacrene D (0.8–7.4 %) and β-caryophyllene (1.4–6.1 %) were also identified (Table 3).

Two components predominate in the EOs from *L. luisieri*: camphor (4.6–49.2 %) and trans-α-necrodyl acetate (13.2–18.1 %). Lavandulol was present at low proportions (5.9–7.9 %). In the HD EO from *L. lanata*, with an IC_{50} of 27.9 μg/ml, lavandulol and camphor were identified at remarkably high proportions (52.6 % and 33.7 %, respectively).

The main components of the genus *Thymus* (*T. vulgaris* and *T. zygis*) were similar in the four EOs extracted with two different methods. Thymol (21.2–43.8 %) and ρ-cymene (8.0–31.7 %) predominate in both plant species, although at higher concentrations in *T. vulgaris*, the most active of both plants (IC_{50} < 25 μg/ml). Other compounds present were γ-terpinene (1.9–9 %) and linalool (3.5–11.6 %) (Table 4).

Four components were identified at high proportions in the EOs of *S. montana* (IC_{50} range from <25–109.2 μg/ml), carvacrol being the most abundant (32.6–41.3 %), followed by ρ-cymene (11.8–18.5), thymol (7.1–17.3 %), and γ-terpinene (11.7–12.4 %).

In the HD extracted EO of *S. sclarea* (IC_{50} = 88.2 μg/ml), a high amount of linalyl acetate (31.2 %), linalool (22.7 %), α-terpineol (11.3 %), neryl acetate (7.5 %), and germacrene D (6.0 %) were identified.

The EOs of *Lavandula intermedia* varieties "Super" and "Abrial" (IC_{50}=111.3–113 in HD EOs and 251.8–271.5 in SD EOs) contain four main components at different proportions: linalool (19.1–39.2 %), linalyl acetate (13–23 %), 1,8-cineole (7.9–20.6 %), and camphor (8.5–16.5 %). At minor proportions β-caryophyllene (5.5 % in *L. intermedia* "Abrial" SD EO) were also identified.

The main components of *O. virens* HD EO, the most active (IC_{50} = 199.4 μg/ml), were γ-terpinene (22.4 %), linalool (13.9 %) and ρ-cymene (12.2 %), while γ-terpinene (15.4 %) and linalool (15.3 %) predominate in the *O. virens* SD EO (IC_{50} = 275.9 μg/ml). Minor components (4.7–6 %) were also detected in *O. virens* HD EO (β-myrcene, carvacrol methyl ether, thymol, and carvacrol), with lower proportions in general in the SD EO.

3.3. Anti-Giardia activity of main components, cytotoxicity and SI

Available pure compounds from the EOs were evaluated against *Giardia* trophozoites (n = 16), most of them were present at concentrations higher than 5 % in some of the active EOs, except three of them that were at concentrations higher than 2.5 % (α-pinene, ocimene, 4-terpinenol). They were also assessed with Vero cells to analyze cytotoxicity (Table 5). Six compounds were active against *Giardia* trophozoites at doses lower than 100 μg/ml, α-pinene, γ-terpinene, caryophyllene oxide, β-caryophyllene, carvacrol and thymol, ordered from higher to lower AG activity (Fig. 1). Of them, two showed cytotoxicity when evaluated at 100 μg/ml, α-pinene and β-caryophyllene. The other four compounds were considered as promising anti-*Giardia* agents, being γ-terpinene the compound with the highest SI obtained (2.4), followed by caryophyllene oxide (SI = 1.8), carvacrol (SI = 1.7), and thymol (SI = 1.3).

3.4. Synergies of pure compounds

Considering the composition of the active EOs and the availability of pure compounds, their synergistic activity was explored by pair combinations of compounds (Fig. 2, supplementary Figures 1 and 2). Compounds representing less than 8 % of the composition and cytotoxic compounds were not considered for the synergies experiment.

The combinations of linalyl acetate or linalool with most of the AG active compounds (camphor, α-terpineol, 1,8-cineol, and γ-terpinene) did not show any synergistic activity (Supplementary Figures 1 and 2).

However, the combinations linalyl acetate with linalool, as well as the combination linalyl acetate with thymol or ρ-cymene displayed synergistic activity since the observed AG activity was significantly higher than the expected activity (sum of the activity of each compound separately) (Fig. 2). The synergistic combinations linalyl acetate with linalool was present in several of the active EOs obtained by HD (*S. sclarea, L. intermedia* "Super" and Aabrial", IC_{50} values from 88.2 to 113 μg/ml).

Synergistic effect was observed when thymol was combined with ρ-cymene or γ-terpinene, and when γ-terpinene was combined with carvacrol. These combinations were present in several of the EOs with lower IC_{50} values, such as *Thymus* and *Satureja* EOs (IC_{50} values from <25 μg/ml to 113 μg/ml).

No synergistic effect was observed with the combination of carvacrol with thymol or ρ -cymene (supplementary Figure 1). These combinations were present in significant amounts in both *S. montana* EOs (IC_{50} ranging <25 μg/ml to 109.2 μg/ml). However, besides a high amount of carvacrol, in both EOs γ-terpinene and thymol are present at amounts higher than 6 %.

In the case of γ-terpinene, high activities (close to 100 % both, in expected and obtained values) were observed when combined with ρ-cymene or linalyl acetate, and for that reason no synergistic activity could be determined (Supplementary Figure 2).

In summary, most of the synergies were observed when the monoterpenoid ester linalyl acetate was combined with monoterpenes or when monoterpenes were combined in pairs. The presence of thymol or ρ-cymene was observed in most of the cases of synergies, and carvacrol and γ-terpinene were observed in others. None of the other compounds evaluated were present in the synergies obtained.

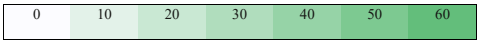
3.5. Morphological changes of γ-terpinene on G. duodenalis trophozoites

Since γ-terpinene was the most active compound against *G. duodenalis*, trophozoite morphology was examined by TEM in non-exposed (Fig. 3 A–C) and exposed (Fig. 3 D–L) parasites. After 60 min of exposure, vacuolization was evident and most trophozoites appeared non-viable (Fig. 3 D–F). Compared with controls, treated trophozoites showed marked alterations, including disrupted plasma membrane and adhesive disk, enlarged periplasmic vacuoles, and cytoplasmic loss.

Table 4
Main components (in percentage) of the EOs with IC_{50} values lower than 200 μg/ml and ordered by retention index. Me: *M. suaveolens*; Tv: *T. vulgaris*; Ll: *L. luisieri*; Sjm: *S. montana*; Lla: *L. lanata*; Tz: *T. zygis*; Ss: *S. sclarea*; Om: *O. majorana*; Lsup: *L. intermedia* "Super"; Lab: *L. intermedia* "Abrial"; Ov: *O. virens*. HD, EOs obtained by hydrodistillation; SD, EOs obtained by steam distillation.

EO / Compound	Me SD	Me HD	Tv HD	Tv SD	Ll SD	Sjm SD	Lla HD	Ll HD	Tz HD	Tz SD	Ss HD	Sjm HD	Lsup HD	Lab HD	Ov HD
IC_{50} (μg/ml)	<25	<25	<25	<25	<25	<25	27.9	32.1	66.9	71.5	88.2	109.2	111.3	113	199.4
α-pinene	0.9	1.1	1.1	1.4	2.6	0.3	0.8	2.8	1.7	0.4	0.1	0.9	0.2	0.4	0.7
camphene	-	-	0.5	1.1	-	0.2	7.0	-	2.1	0.5	0.6	0.5	0.2	0.6	0.3
sabinene	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	2.7
β-pinene	0.7	1.8	0.5	0.5	-	0.2	0.3	-	0.4	0.2	-	0.4	-	0.7	0.5
β-myrcene	1.2	0.5	1.6	0.7	-	1.1	-	-	1.5	0.8	2.0	1.4	1.1	1.4	6.0
α-terpinene	-	-	1.4	1.0	-	1.9	-	-	1.6	0.5	-	2.6	-	-	3.1
ρ-cymene	-	-	22.3	31.7	-	11.8	0.1	-	18.3	8	-	18.5	-	0.3	12.2
1,8-cineole	-	1.3	2.3	2.2	2.0	0.9	3.6	0.9	-	-	-	0.9	8.8	20.6	-
ocimene	-	-	-	-	-	-	-	-	-	-	0.4	-	-	1.0	2.7
γ-terpinene	-	-	5.7	2.8	-	12.4	-	-	9.0	1.9	-	11.7	-	-	22.4
D-fenchone	-	-	-	-	1.3	-	-	2.5	-	-	-	-	-	-	-
linalool	-	1.2	3.8	3.5	2.2	1.1	0.5	1.1	5.4	11.6	22.7	1.3	39.2	27.8	13.9
β-thujone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-thujone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
camphor	-	0.3	0.5	0.9	4.6	0.1	33.7	49.2	1.0	2.7	0.2	0.1	8.5	16.5	0.1
borneol	0.4	0.6	1.0	2.0	1.6	1.3	-	1.3	6.3	2.5	-	2.0	4.6	4.0	0.7
trans-bornyl acetate	-	-	-	-	-	-	0.1	-	-	-	-	0.2	-	-	-
4-terpinenol	0.3	-	1.5	0.8	-	0.9	-	-	0-8	1.4	-	1.5	-	0.7	2.6
α-terpineol	-	-	0.3	0.2	-	0.2	0.2	-	0.1	0.6	11.3	0.2	5.2	3.4	0.3
carvacrol methyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.7
linalyl acetate	-	-	-	0.1	-	-	-	-	-	-	31.2	-	21.5	13	0.2
trans-α-necrodyl acetate	-	-	-	-	18.1	-	-	13.2	-	-	-	-	-	-	-
lavandulol	-	-	-	-	7.9	-	52.6	5.9	-	-	-	-	-	3.2	-
thymol	-	-	43.8	28.1	-	7.1	-	-	39.5	21.2	-	17.3	-	-	6.6
carvacrol	-	-	2.42	2.3	-	41.3	-	-	5.0	1.4	-	32.6	-	-	4.7
neryl acetate	-	-	-	-	-	-	-	-	0.7	-	7.5	-	1.0	0.5	-
β-caryophyllene	6.1	1.4	2.4	5.8	3.7	6.1	0.6	0.1	1.4	4.0	3.0	2.1	0.5	0.9	2.1
α-humulene	0.8	-	0.1	0.3	-	-	-	-	-	-	0.2	0.1	-	-	0.2
Lavandulyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	2.6	-	-
germacrene D	7.4	0.8	0.1	0.1	-	-	-	-	-	-	6.0	-	0.1	-	0.4
spatulenol	-	-	-	-	-	0.2	-	-	0.2	-	0.2	-	-	-	1.7
α-bisabolol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
caryophyllene oxide	-	-	1.1	1.7	1.2	1.4	-	0.1	0.9	0.8	0.6	1.0	0.2	0.7	1.1
viridiflorol	0.5	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
epimanol	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-
Piperitenone	20.8	53.4	-	-	-	-	-	-	-	-	-	-	-	-	-
Piperitenone oxide	37.0	22.8	-	-	-	-	-	-	-	-	-	-	-	-	-
Limonene	6.6	5.6	-	-	-	-	-	-	1.2	0.5	-	-	-	-	1.1

-: not detected. Percentage of each compound according to color intensity:



-: not detected. Percentage of each compound according to color intensity:

Table 5
IC_{50} (μg/ml), CC_{50} (μg/ml) and SI values of the tested main components from the EOs.

Pure compound	IC_{50} (95 % CI)	CC_{50} (95 % CI)	SI
1,8-cineole	> 100	> 100	1
Thymol	75.3 (71.2–79.7)	> 100	1.3
Carvacrol	59.3 (52.5–67)	> 100	1.7
ρ-cymene	> 100	> 100	1
γ-terpinene	41.2 (35.8–47.4)	> 100	2.4
Linalyl acetate	> 100	> 100	1
Linalool	> 100	> 100	1
α-terpineol	> 100	> 100	1
Caryophyllene oxide	57.1 (52.7–61.8)	> 100	1.8
α-pinene	25.2 (20.2–31.6)	88.9 (88–89.7)	3.5
Camphor	> 100	> 100	1
Borneol	> 100	> 100	1
D-fenchone	> 100	> 100	1
β-caryophyllene	57.3 (50.1–65.5)	60.8 (56.9–65)	0.7
α and β thujone	> 100	> 100	1
4-terpinenol	> 100	> 100	1
Camphene	> 100	74.8 (66.3–84.2)	0.8
Limonene	> 100	> 100	1
Ocimene	> 100	> 100	1
Metronidazole	4.4 (3.5–5.5)	> 100	22.7

IC_{50} (μg/ml), concentration needed to produce 50 % trophozoite mortality; CC_{50} (μg/ml), concentration needed to produce 50 % Vero cell mortality; SI, Selectivity index (CC_{50}/IC_{50}).

Partial flagella internalization was occasionally observed. After 24 h (Fig. 3 H–L), fewer trophozoites remained altered, while others resembled controls (Fig. 3 G), though some retained distinctive changes such as modified vacuole systems and swollen adhesive disks.

4. Discussion

Natural products derived from Lamiaceae and Asteraceae plants are among the most widely used remedies for gastrointestinal disorders in Mediterranean traditional medicine, including diarrhea and infections of viral, bacterial or parasitic origin (Rahman et al., 2016; Redouan et al., 2022). Several plant-derived natural products have been evaluated against *Giardia* spp., with Lamiaceae (30 % of the studies) and Asteraceae (13,5 %) being the most frequently investigated plants (Alnomasy et al., 2021). In our study, Lamiaceae EOs showed strong AG activity, particularly those from *Mentha*, *Thymus*, *Lavandula*, *Satureja* and *Origanum* genera.

M. suaveolens EOs exhibit strong AG activity (IC_{50} < 25 μg/ml), comparable to no-toxic extracts of *M. x piperita* (Vidal et al., 2007), though their composition was not reported. Ethanolic extracts of *Mentha longifolia*, with menthol as the main component, also exhibited good activity without toxicity (El-Badry et al., 2010). In our study, piperitenone and piperitenone oxide predominate in the composition of both EOs. Unfortunately, these compounds could not be assessed against *Giardia* trophozoites due to lack of availability.

Essential oils of *T. vulgaris* (IC_{50} < 25 μg/ml), *T. zygis* (IC_{50} = 66.9–71.5 μg/ml), and *O. virens* (IC_{50} = 199.4 μg/ml) showed notable AG activity in the present study. Similar results were reported for EOs of *Thymus* and *Origanum* species, including *T. capitata*, *T. zygis silvestris*, and *O. virens* (IC_{50} = 71–185 μg/ml) and other *Origanum* species (IC_{50} = 22–108 μg/ml) (Machado et al., 2010a; Andrade-Ochoa et al., 2021). In these studies, thymol, γ-terpinene, ρ-cymene, and carvacrol, were identified as the major constituents, consistent with our findings in both genera.

In our study, *Satureja* EOs showed strong AG activity with IC_{50} values from < 25–109.2 μg/ml depending on the extraction method. Both oils were rich in carvacrol, thymol, γ-terpinene, and ρ-cymene, compounds active against *Giardia* trophozoites. Synergism between carvacrol and γ-terpinene may explain the high AG activity observed in S. *montana* EOs. Similar composition with anti-bacterial properties has been reported for *S. montana* EO (Cavar et al., 2008), and other studies confirmed activity of *Satureja* EOs against *T. gallinae*, *Leishmania* spp., *Acanthamoeba* spp., *Trypanosoma* spp., fungi and bacteria (Monzote et al., 2012; Jafari et al., 2016; Bailen et al., 2022).

Essential oils of *L. luisieri* and *L. lanata* HD EO exhibit strong AG activity (IC_{50} = 25–32.1 μg/ml), while *L. intermedia* EOs displayed moderate AG activity (*L. intermedia* Ssuper" HD EO, IC_{50}= 111.3 μg/ml and *L. intermedia* "Abrial" HD EO, IC_{50} = 113 μg/ml). Few studies have assessed the anti-parasitic activity of *Lavandula* spp. against *Giardia* spp., although *L. angustifolia* and *L. intermedia* EOs were previously reported as active (Moon et al., 2006). Activity has also been demonstrated against *Trichomonas* and *Hexamita* spp. (Moon et al., 2006; Bailén et al., 2022). Differences among the oils may reflect oil composition or compound synergies: *L. luisieri* EOs are rich in trans-α-necrodyl acetate and camphor, whereas in *L. intermedia* oils, linalool and linalyl acetate predominate, with lower amounts of camphor and 1,8-cineole (Table 3). However, we could not assess trans-α-necrodyl acetate against *Giardia* trophozoites due to lack of availability.

Extracts and components of *Salvia* species have previously shown AG activity (Calzada et al., 2015; Shaverdi et al., 2024). In our conditions, *S. sclarea* HD EO exhibits good AG activity (IC_{50} = 88.2 μg/ml), with linalyl acetate and linalool as major constituents, similar to *L. intermedia* EOs of the present study. Other reports indicate *Salvia* EOs are also active against other protozoa, such as *Plasmodium* spp. (Monzote et al., 2012). In *S. mirzayanii* EO, with good AG activity, linalool and linalyl acetate were present at 12.7 and 23.9 μg/ml, respectively (Shaverdi et al., 2024), proportions comparable to those found in our study.

The main antiprotozoal compounds in essential oils are monoterpenoids (e.g. linalool, 4-terpinenol, thymol, carvacrol, citral, limonene, α-pinene, γ-terpinene, α-phellandrene, and *p*-cymene), sesquiterpenes (e.g. β-caryophyllene, nerolidol, α-copaene, cyperene, and germacrene D), and phenylpropanoids (e.g. eugenol, methyl chavicol, and cinnamaldehyde) (Monzote et al., 2012). In our study, the less cytotoxic and most active against *Giardia* trophozoites were γ-terpinene, thymol, carvacrol, and α-pinene, and caryophyllene oxide, all with good minimum SI, although α-pinene shows slight cytotoxicity in Vero cells. Similar activity of monoterpenoids was reported against *T. gallinae* (Bailén et al., 2022). Our findings on terpenes agree with recent studies, where thymol and carvacrol were active against *Giardia* trophozoites with IC_{50} values of 21.4 μg/ml and from 31.9 to 50 μg/ml, respectively (Andrade-Ochoa et al., 2021; Machado et al., 2025).

Beta-caryophyllene was identified as an active compound in the present study and in previous work with *T. gallinae* (Bailén et al., 2022). It has also been reported in *A. conyzoides* extracts at close to 20 %, although chromenes (precone I & II) were more abundant but remained untested against *Giardia* (Pintong et al., 2020). Due to its cytotoxicity in Vero cells observed here, β-caryophyllene was excluded for further evaluation.

The chemical structures of the terpenes may influence their activity. Oxidation of the double bound 14,5 of β-caryophyllene to produce caryophyllene oxide reduced cytotoxicity. The presence and position of hydroxyl groups on the aromatic ring of monoterpenes also influence their AG activity, as evidenced by the higher activity of thymol and carvacrol compared with the weaker effect of ρ-cymene. All compounds complied with Lipinski's rule of five, supporting their potential for orally administration.

Compounds that are present in high proportions in the EOs with lower IC_{50} values, such as piperitenone and piperitenone oxide from *Mentha suaveolens* extracts, and trans-α-necrodyl acetate and lavandulol in *Lavandula* species could not be assessed by us due to lack of availability. These constituents may contribute to the AG activity observed, and piperitenone oxide has previously shown significant anti-protozoal effects against *Trypanosoma* (Guardo et al., 2017).

Our study suggests that synergistic effects may contribute to the activity of some EOs, especially those rich in monoterpenoids. Synergism was observed in combinations such as γ-terpinene with carvacrol or

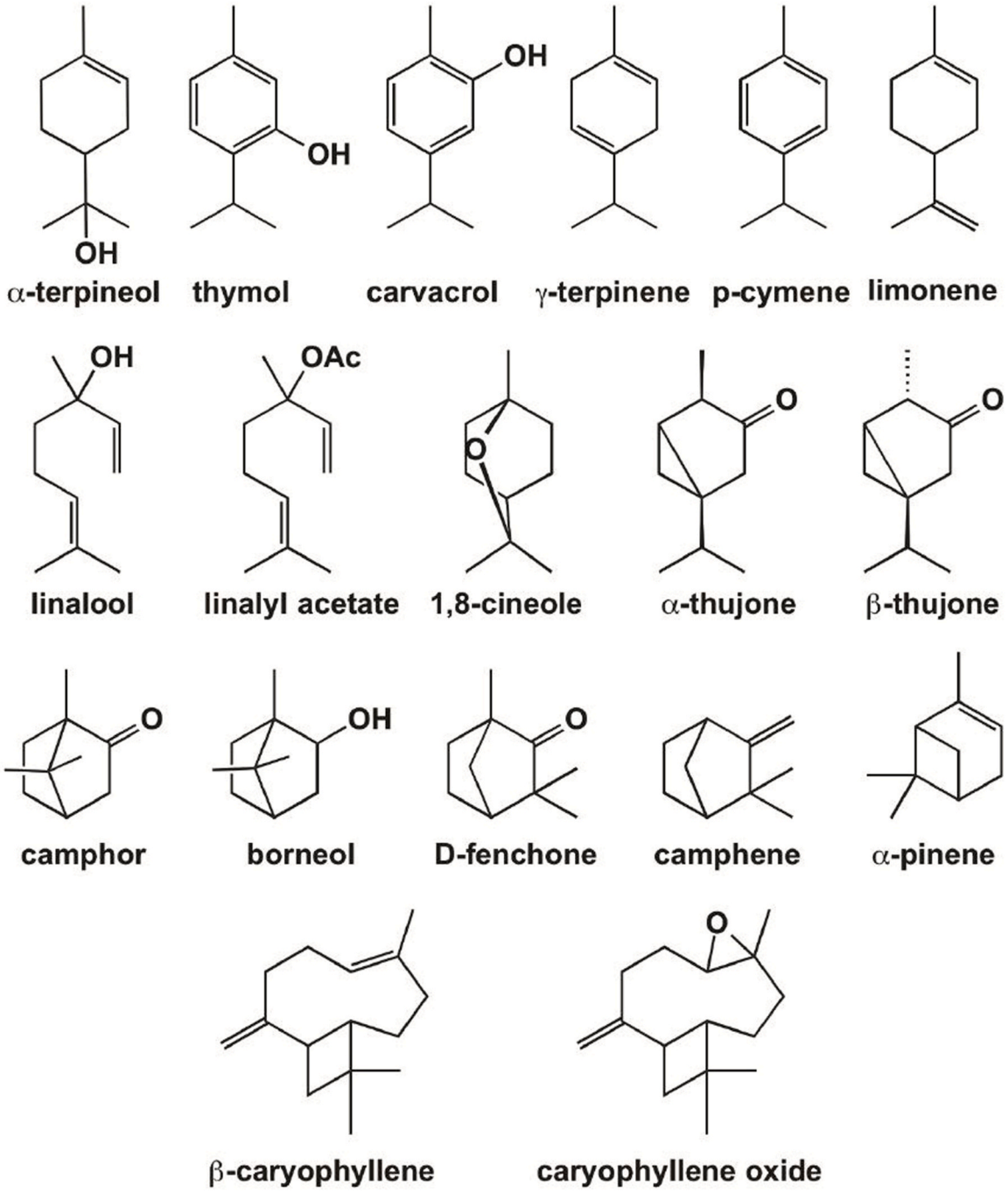


Fig. 1. Chemical structure of the major components identified from the EOs employed in this study.

thymol, thymol with ρ-cymene, and linalyl acetate with linalool, ρ-cymene, or thymol.

The synergistic combinations linalyl acetate and linalool was present in the HD *L. intermedia* "Super and "Abrial (IC_{50} = 111.3 and 113 μg/ml, respectively), and HD *S. sclarea* (IC_{50} = 88.2 μg/ml), which could explain the AG activity. In contrast, camphor + 1,8-cineol showed no synergism, and 1,8-cineol has been reported to act antagonistically (Guardo et al., 2017). Linalool was also detected in *Ferula macrecolea* EO, which exhibited good AG activity (Salimikia et al., 2025).

No synergy was detected between thymol and carvacrol, despite their abundance in both *S. montana* EOs. The observed AG effect may instead result from γ-terpinene and ρ-cymene in combination with thymol or carvacrol. However, the expected and observed values of γ-terpinene and ρ-cymene combination approaches 100 %, complicating the analysis of their synergistic effects.

The most consistent synergies involved four monoterpenes. Thymol + γ-terpinene with slight synergistic effect, appeared in EOs of *S. montana* (IC_{50} = 25–109.2 μg/ml), *T. zygis* (IC_{50} = 66.9–71.5 μg/ml), and *O. virens* (IC_{50} = 199.4–275.9 μg/ml). Thymol and ρ-cymene also displayed slight synergism and they were present in EOs of *T. vulgaris* (IC_{50} < 25 μg/ml), *S. montana* (IC_{50} = 25–109.2 μg/ml), and *T. zygis* (IC_{50} = 66.9–71.5 μg/ml). The γ-terpinene and ρ-cymene combination yielded strong AG effects, and they appeared in EOs of *T. vulgaris, T. zygis*, and *S. montana*, with low IC_{50} values, and in the less AG active EOs of *O. majorana* (HD EO) and *O. virens* (SD EO). Finally, γ-terpinene and carvacrol showed good synergism and they were present in *S. montana*

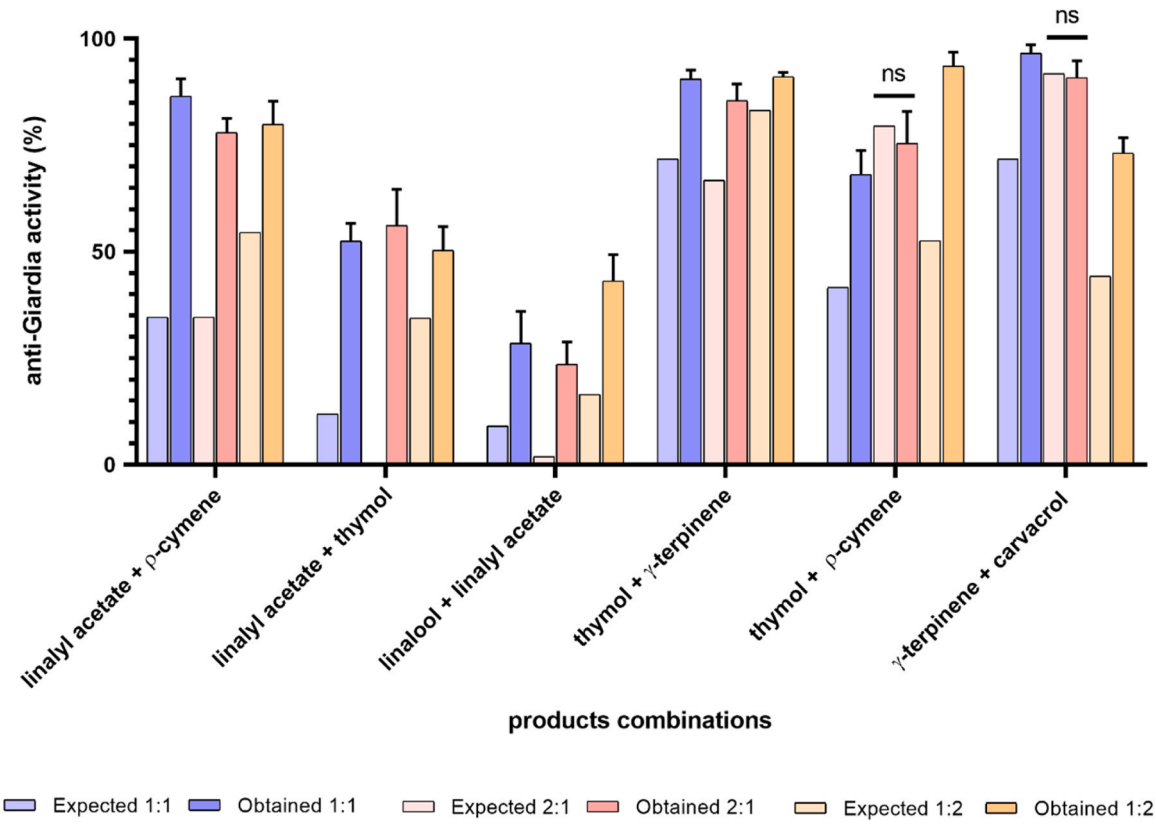


Fig. 2. Activity of compounds combined in pairs from the most active AG EOs that showed synergistic activity. Significant differences ($p < 0.05$) were observed in all experiments (expected vs. observed), except for γ-terpinene + carvacrol (2:1) and thymol + p-cymene (2:1). n.s. non- significant.

EOs (IC_{50} = 25–109.2 μg/ml).

Few studies of synergies against *Giardia* were carried out, but they aligned with our results, whit thymol, carvacrol and γ-terpinene present in several EOs, while 1,8-cineole appeared in antagonistic combinations (Machado et al., 2010a; Guardo et al., 2017; Andrade-Ochoa et al., 2021). Interestingly, 1,8 cineol has been described as AG by other authors (Masoori et al., 2024), though it shows no relevant AG activity in our assay (Table 5) and was often present in EOs with high IC_{50} values (Supplementary Table 1). Besides the positive effect of synergies, the volatility, low stability and lipophilic nature of these monoterpenes can help to explain why some EOs had lower IC_{50} than the pure compounds (Hajibonabi et al., 2023).

Terpenoid-rich phenolic extracts appear to act against *Giardia* trophozoites through a multi-hit mechanism involving direct membrane damage and interference in microtubule dynamics (Palomo-Ligas et al., 2022; Machado et al., 2025). Ventral disc damage may further reduce parasite attachment to the intestinal epithelium, facilitating clearance in vivo via peristalsis.

In our study, γ-terpinene induced significant marked morphological changes within 60 min, including plasma membrane disruption, vacuolization, ventral disc deformation, cytoplasmic disorganization, and flagellar internalization. Severely affected trophozoites were only observed at 60 min, and few were detected at 24 h, a time point at which only surviving trophozoites that had multiplied were present. The volatile nature of the compound could probably lead to a less prolonged effect. Similar effects have been described with methanolic extracts of *Lippia* spp., rich in structurally related terpenoids, such as thymol and *p*-cymene (Ponce-Macotela et al., 2006). A similar effect was also observed using extracts from commercial spices with eucalyptol, cumin aldehyde, and anethole (Andrade-Ochoa et al., 2021). Monoterpene phenols (thymol and carvacrol) and their precursors (*p*-cymene and γ-terpinene), present in plants such as *Thymbra capitata*, *O. virens*, *T. zygis*, and *Lippia graveolens*, have been shown to permeabilize trophozoite plasma membranes within minutes, leading to osmotic imbalance and massive vacuolization (Machado et al., 2010a,b; Machado et al., 2011; Machado et al., 2025). These lesions have been attributed to the insertion of lipophilic monoterpenes into the lipid bilayer, increasing membrane fluidity and destabilization (Machado et al., 2010a; Andrade-Ochoa et al., 2021; Machado et al., 2025).

Several natural products, including curcumin, galacto-glycerolipids from *Oxalis corniculata*, garlic extracts (allyl alcohol and diallyl thiosulfinate), dichloromethane and methanolic extracts of *Mentha* × *piperita*, and ethanolic extracts from *Ageratum conyzoides*, have produced similar cytoplasmic and membrane alterations in *Giardia* trophozoites (Harris et al., 2000; Pérez-Arriaga et al., 2006; Vidal et al., 2007; Manna et al., 2010; Argüello-García et al., 2018; Pintong et al., 2020). Beyond membranolysis, cell damage may involve tubulin interactions, reducing α-tubulin abundance and disrupting microtubule organization, particularly in the ventral disc (Palomo-Ligas et al., 2022). Curcumin, for instance, binds at the tubulin dimer interface, near the colchicine/vinblastine binding site, disrupting cytoskeletal structures including the ventral disc and flagella (Gutiérrez-Gutiérrez et al., 2017).

The ventral disc alterations observed in γ-terpinene-treated trophozoites in this study is consistent with microtubule destabilization. However, other studies reported no visible damage to cytoskeletal structures or the adhesive disc after treatment with ethanolic extracts of *Lippia* or allicin from garlic, suggesting that cytoskeletal disruption may depend on the compound or its concentration (Ponce-Macotela et al., 2006; Argüello-García et al., 2018).

5. Conclusions

The EOs of *Lavandula*, *Mentha*, *Thymus,* and *Satureja* genera, plants frequently employed in traditional medicine to treat gastrointestinal

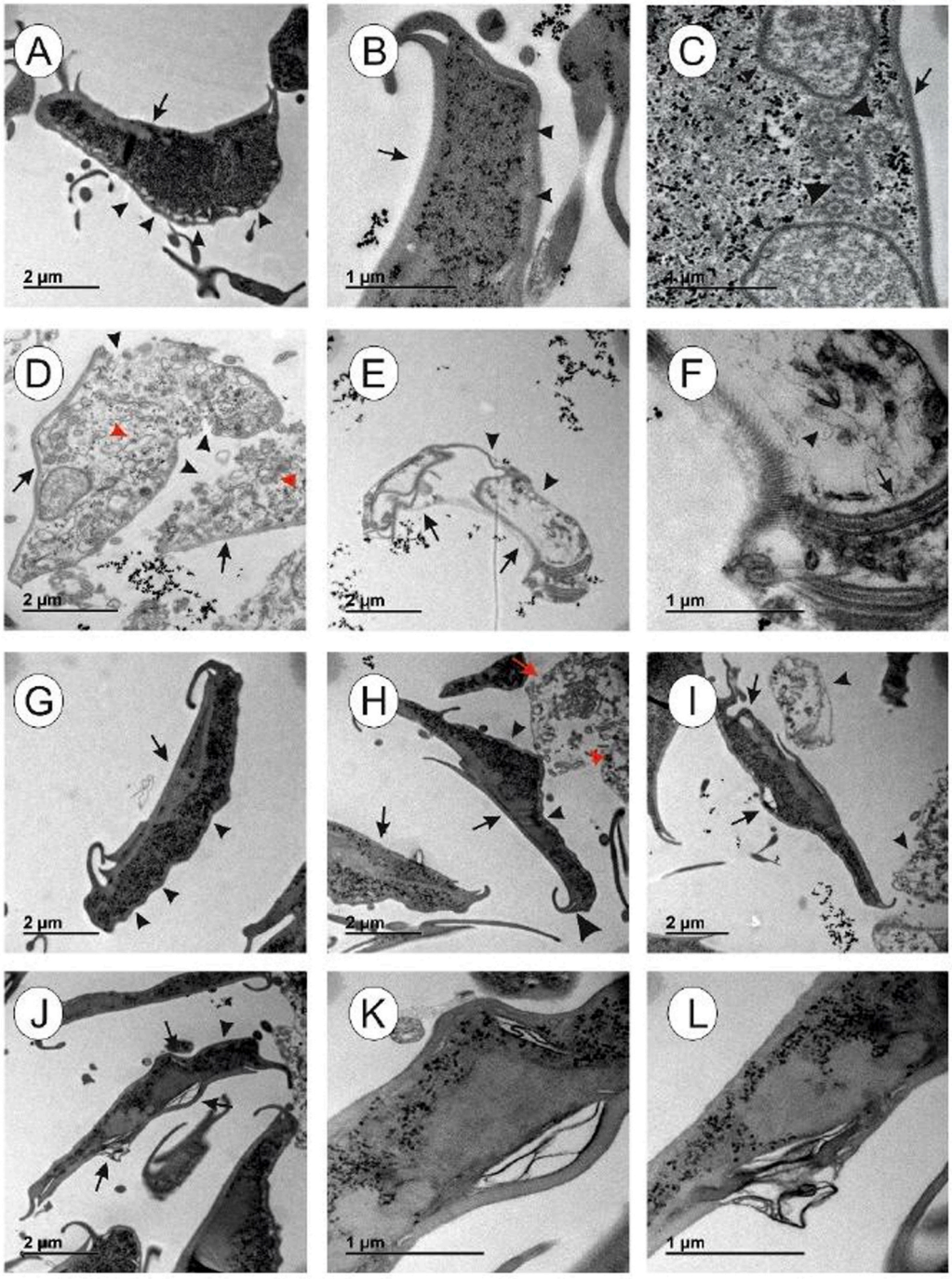


Fig. 3. Transmission electron microscopy of *G. duodenalis* trophozoites under control conditions (1 h: A–C; 24 h: G) and after γ-terpinene exposure (1 h: D–F; 24 h: H–L). Control cells show normal morphology, including intact adhesive disks, vesicles beneath the plasma membrane, granular cytoplasm, and typical flagella and nuclei. γ-Terpinene-treated trophozoites display progressive structural damage, including membrane disruption, cytoplasmic disorganization, vacuolization, swollen regions, altered adhesive disks, and partial flagella internalization. Panels K–L show details of the damaged areas.

disorders, have shown good in vitro AG activity and are, therefore, promising candidates as natural antigiardial agents. Nevertheless, *in vivo* studies have to be performed to assure their efficacy. The most active compounds identified in the present study, γ-terpinene, thymol, carvacrol and caryophyllene oxide adheres to the Lipinski's rule of five, are non-toxic, and the first three of them have been authorized for use as additives for animals food (Commission Implementing Regulation, 2025), which makes them ideal for alternative or complementary treatment of giardiasis, and other gastrointestinal protozoosis. Prospective studies including recultivation of trophozoites to differentiate between killing effects versus giardiastatic effects, together with in vivo testing would help to validate findings of this study.

Authors contribution

Conceptualization and design: MB, AG and MTG. Acquisition of data: SMH, SAF, MJI, JAD, FP-G, JNR and IAC. Analysis and interpretation of data: MB, AG, MTG, SMH, SAF, MJI, JAD, FP-G, JNR and IAC. Funding acquisition: AG and MTG. Draft of the article: MB and MTG. Critical revision for important intellectual content: MB, AG and MTG. Final

approval: all the authors.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2026.110702.

References

Alnomasy, S., Al-Awsi, G.R.L., Raziani, Y., Albalawi, A.E., Alanazi, A.D., Niazi, M., Mahmoudvand, H., 2021. Systematic review on medicinal plants used for the treatment of *Giardia* infection. Saudi J. Biol. Sci. 28, 5391–5402. https://doi.org/10.1016/j.sjbs.2021.05.069.

Andrade-Ochoa, S., Chacón-Vargas, K.F., Sánchez-Torres, L.E., Rivera-Chavira, B.E., Nogueda-Torres, B., Nevárez-Moorillón, G.V., 2021. Differential antimicrobial effect of essential oils and their main components: insights based on the cell membrane and external structure. Membranes 1, 405. https://doi.org/10.3390/membranes11060405.

Anthony, J.P., Fyfe, L., Smith, H., 2005. Plant active components – a resource for antiparasitic agents? Trends Parasitol. 21, 462–468. https://doi.org/10.1016/j.pt.2005.08.004.

Argüello-García, R., de la Vega-Arnaud, M., Loredo-Rodríguez, I.J., Mejía-Corona, A.M., Melgarejo-Trejo, E., Espinoza-Contreras, E.A., Fonseca-Liñán, R., González-Robles, A., Pérez-Hernández, N., Ortega-Pierres, M.G., 2018. Activity of thioallyl compounds from garlic against *Giardia duodenalis* trophozoites and in experimental giardiasis. Front. Cell. Infect. Microbiol 8, 353. https://doi.org/10.3389/fcimb.2018.00353.

Arroyo Díaz, J.E., Gómez Muñoz, M.T., Martínez-Díaz, R.A., González-Coloma, A., 2023. Adaptation of the colorimetric MTT assay for evaluating activity against *Giardia duodenalis*. Rev. Arg. Parasitol. 12, 7–14. ISSN 2313-9862.

Aziz, Z.A.A., Ahmad, A., Setapar, S.H.M., Karakucuk, A., Azim, M.M., Lokhat, D., et al., 2018. Essential oils: extraction techniques, pharmaceutical and therapeutic potential-a review. Curr. Drug Metab. 19, 1100–1110. https://doi.org/10.2174/1389200219666180723144850.

Bailén, M., Díaz-Castellanos, I., Azami-Conesa, I., Alonso Fernández, S., Martínez-Díaz, R.A., Navarro-Rocha, J., Gómez-Muñoz, M.T., González-Coloma, A., 2022. Anti-*Trichomonas gallinae* activity of essential oils and main compounds from Lamiaceae and Asteraceae plants. Front. Vet. Sci. 9, 981763. https://doi.org/10.3389/fvets.2022.981763.

Bénéré, E., da Luz, R.A.I., Vermeersch, M., Cos, P., Maes, L., 2007. A new quantitative *in vitro* microculture method for *Giardia duodenalis* trophozoites. J. Microbiol. Methods 71, 101–106.

Calzada, F., Bautista, E., Yépez-Mulia, L., García-Hernandez, N., Ortega, A., 2015. Antiamoebic and Antigiardial Activity of Clerodane Diterpenes from Mexican *Salvia* Species Used for the Treatment of Diarrhea. Phytother. Res. 29, 1600–1604. https://doi.org/10.1002/ptr.5421.

Carter, E.R., Nabarro, L.E., Hedley, L., Chiodini, P.L., 2018. Nitroimidazole-refractory giardiasis: a growing problem requiring rational solutions. Clin. Microbiol. Infect. 24, 37–42. https://doi.org/10.1016/j.cmi.2017.05.028.

Cavar, S., Maksimovic, M., Šolic, M.E., Jerkovi -Mujki, A., Bešta, R., 2008. Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. Food Chem. 111, 648–653. https://doi.org/10.1016/j.foodchem.2008.04.033.

Commission Implementing Regulation (EU) 2024/1989 of 22 July 2024 concerning the authorisation of undec-10-enal, terpineol acetate, d,l-borneol, l-carvone, d-camphor, d,l-isobornyl acetate, 3-propylidenephthalide, phenylacetic acid, methyl salicylate, thymol, carvacrol, benzothiazole, terpinolene, d,l-isoborneol, trans-menthone, d,l-bornyl acetate, 3-butylidenephthalide, phenylacetaldehyde, phenethyl acetate, phenethyl phenylacetate, methyl phenylacetate, ethyl phenylacetate, isobutyl phenylacetate, 3-methylbutyl phenylacetate, 2-methoxyphenol, 2-methoxy-4-methylphenol, 4-ethylguaiacol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 2-methylphenol, 4-methylphenol, 2,6-dimethoxyphenol, phenol, 2,6-dimethylphenol, 2-isopropylphenol, benzene-1,3-diol, alpha-phellandrene, alpha-terpinene, gamma-terpinene and l-limonene as feed additives for all animal species and amending Implementing Regulation (EU) 2018/245 as regards the terms of authorisation of d,l-isomenthone as a feed additive for all animal species ⟨https://eur-lex.europa.eu/eli/reg_impl/2024/1989/oj/eng⟩ (accessed on 4 November 2025).

Ciuca, L., Pepe, P., Bosco, A., Caccio, S.M., Maurelli, M.P., Sannella, A.R., Vismarra, A., Cringoli, G., Kramer, L., Rinaldi, L., Genchi, M., 2021. Effectiveness of Fenbendazole and Metronidazole Against *Giardia* Infection in Dogs Monitored for 50-Days in Home Conditions. Front. Vet. Sci. 8, 626424. https://doi.org/10.3389/fvets.2021.626424.

Drake, J., Sweet, S., Baxendale, K., Hegarty, E., Horr, S., Friis, H., Goddu, T., Ryan, W.G., von Samson-Himmelstjerna, G., 2022. Detection of *Giardia* and helminths in Western Europe at local K9 (canine) sites (DOGWALS Study). Parasites Vectors 15, 311. https://doi.org/10.1186/s13071-022-05440-2.

El-Badry, A.A., Al-Ali, K.H., El-Badry, Y.A., 2010. Activity of *Mentha longifolia* and *Ocimum basilicum* against *Entamoeba histolytica* and *Giardia duodenalis*. Sci. Parasitol. íca 11, 109–117. ISSN (Print): 1582-1366. CABI Record Number: 20103327855.

Es-Safi, I., Mechchate, H., Amaghnouje, A., Jawhari, F.Z., Bari, A., Cerruti, P., Avella, M., Grafov, A., Bousta, D., 2020. Medicinal plants used to treat acute digestive system problems in the region of Fez-Meknes in Morocco: an ethnopharmacological survey. Ethnoboth. Res. Appl. 20, 1–14. https://doi.org/10.32859/era.20.25.1-14. https://ethnobotanyjournal.org/index.php/era/article/view/2201.

European Centre for Disease Prevention and Control. Giardiasis (lambliasis). Annual Epidemiological Report for 2019. Stockholm. ⟨https://www.ecdc.europa.eu/en/publications-data/giardiasis-lambliasis-annual-epidemiological-report-2019/⟩ 2022 (accessed 4 November 2025).

European Pharmacopoeia. ⟨http://www.edqm.eu/en/Homepage-628.html⟩ (accessed 4 November 2025).

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110–140. https://doi.org/10.1128/cmr.00033-10.

González-Coloma, A., Guadaño, A., de, Inés, C., Martínez-Díaz, R., Cortes, D., 2002. Selective action of acetogenin mitochondrial complex I inhibitors. Z. Naturforsch. C. J. Biosci. 57, 1028–1034. https://doi.org/10.1515/znc-2002-11-1213.

Guardo, N.I., Paula, S., González-Coloma, A., Burillo, J., Martínez-Díaz, R.A., 2017. Trypanocidal effects of essential oils from selected medicinal plants synergy among the main components. Nat. Prod. Commun. 12, 709–712. https://doi.org/10.1177/1934578X1701200516.

Gutiérrez-Gutiérrez, F., Palomo-Ligas, L., Hernández-Hernández, J.M., Pérez-Rangel, A., Aguayo-Ortiz, R., Hernández-Campos, A., Castillo, R., González-Pozos, S., Cortés-Zárate, R., Ramírez-Herrera, M.A., Mendoza-Magaña, M.L., Castillo-Romero, A., 2017. Curcumin alters the cytoskeleton and microtubule organization on trophozoites of *Giardia lamblia*. Acta Trop. 172, 113–121. https://doi.org/10.1016/j.actatropica.2017.04.027.

Harris, J.C., Plummer, S., Turner, M.P., Lloyd, D., 2000. The microaerophilic flagellate *Giardia intestinalis*: *Allium sativum* (garlic) is an effective antigiardial. Microbiol 146, 3119–3127. https://doi.org/10.1099/00221287-146-12-3119.

Hajibonabi, A., Yekani, M., Sharifi, S., Nahad, J.S., Dizaj, S.M., Menar, M.Y., 2023. Antimicrobial activity of nanoformulations of carvacrol and thymol: new trend and applications. OpenNano 13, 100170. https://doi.org/10.1016/j.onano.2023.100170.

Jamila, F., Mostafa, E., 2014. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. ISSN 0378-8741 J. Ethnopharmacol. 154 (1), 76–87. https://doi.org/10.1016/j.jep.2014.03.016.

Jafari, F., Ghavidel, F., Zarshenas, M.M., 2016. A Critical Overview on the Pharmacological and Clinical Aspects of Popular *Satureja* Species. J. Acupunct. Meridian Stud. 9, 118–127. https://doi.org/10.1016/j.jams.2016.04.003.

Julio, L.F., González-Coloma, A., Burillo, J., Diaz, C.E., Andrés, M.F., 2017. Nematicidal activity of the hydrolate byproduct from the semi industrial vapor pressure extraction of domesticated *Artemisia absinthium* against *Meloidogyne javanica*,. Crop Prot. 94, 33–37. https://doi.org/10.1016/j.cropro.2016.12.002.

Machado, M., Dinis, A.M., Salgueiro, L., Cavaleiro, C., Custódio, J.B.A., Do Céu Sousa, M., 2010a. Anti *Giardia* activity of phenolic-rich essential oils: effects of *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *sylvestris*, and *Lippia graveolens*

on trophozoites growth, viability, adherence, and ultrastructure. Parasitol. Res. 106, 1205–1215. https://doi.org/10.1007/s00436-010-1800-7.

Machado, M., Sousa, M.D.C., Salgueiro, L., Cavaleiro, C., 2010b. Effects of essential oils on the growth of *Giardia lamblia* trophozoites. Nat. Prod. Commun. 5, 137–141. PMID: 20184039.

Machado, M., Dinis, A.M., Salgueiro, L., Custódio, J.B.A., Cavaleiro, C., Sousa, M.C., 2011. Anti-*Giardia* activity of *Syzygium aromaticum* essential oil and eugenol: effects on growth, viability, adherence and ultrastructure. Exp. Parasitol. 127, 732–739. https://doi.org/10.1016/j.exppara.2011.01.011.

Machado, M., Silva, A., Linhares, R., Cavaleiro, C., Sousa, M.C., 2025. Volatile compounds as upcoming antigiardial agents: in vitro action of carvacrol, thymol, and p-cymene on *Giardia lamblia* trophozoites. Pharmaceutics 17, 1380. https://doi.org/10.3390/pharmaceutics17111380.

Manna, D., Dutta, P.K., Achari, B., Lohia, A., 2010. A novel galacto-glycerolipid from *Oxalis corniculata* kills *Entamoeba histolytic*a and *Giardia lamblia*. Antimicrob. Agents Chemother. 54, 4825–4832. https://doi.org/10.1128/AAC.00546-10.

Masoori, L., Khudair Khalaf, A., Ezzatkhah, F., Balaña-Fouce, R., Mahmoudvand, H., 2024. Promising effects of 1,8 Cineole to control *Giardia lamblia* infection: targeting the inflammation, oxidative stress, and infectivity. ISSN 0001-706X Acta Trop. 255, 107201. https://doi.org/10.1016/j.actatropica.2024.107201.

Mateo, M., Montoya, A., Bailo, B., Köster, P.C., Dashti, A., Hernández-Castro, C., Saugar, J.M., Matas, P., Xiao, L., Carmena, D., 2023. Prevalence and public health relevance of enteric parasites in domestic dogs and cats in the region of Madrid (Spain) with an emphasis on *Giardia duodenalis* and *Cryptosporidium* sp. Vet. Med. Sci. 9 (6), 2542–2558. https://doi.org/10.1002/vms3.1270.

Millonig, G., 1961. Advantages of a phosphate buffer for OsO4 solutions in fixation. J. Appl. Phys. 32, 1637. https://doi.org/10.1063/1.1728411.

Monzote, L., Alarcón, O., Setzer, W.N., 2012. Antiprotozoal activity of essential oils. Agric. Conspec. Sci. 77, 167–175.

Moon, T., Wilkinson, J.M., Cavanagh, H.M.A., 2006. Antiparasitic activity of two *Lavandula* essential oils against *Giardia duodenalis*, *Trichomonas vaginalis* and *Hexamita inflata*. Parasitol. Res. 99, 722–728. https://doi.org/10.1007/s00436-006-0234-8.

Nabarro, L.E., Lever, R.A., Armstrong, M., Chiodini, P.L., 2015. Increased incidence of nitroimidazole-refractory giardiasis at the Hospital for Tropical Diseases, London: 2008–2013. Clin. Microbiol. Infect. 21, 791–796.

Palomo-Ligas, L., Estrada-Camacho, J., Garza-Ontiveros, M., Vargas-Villanueva, J.R., Gutiérrez-Gutiérrez, F., Nery-Flores, S.D., Cañas Montoya, J.A., Ascacio-Valdés, J., Campos-Muzquiz, L.G., Rodriguez-Herrera, R., 2022. Polyphenolic extract from *Punica granatum* peel causes cytoskeleton-related damage on *Giardia lamblia* trophozoites in vitro. PeerJ 10, e13350. https://doi.org/10.7717/peerj.13350.

Pérez-Arriaga, L., Mendoza-Magaña, M.L., Cortés-Zárate, R., Corona-Rivera, A., Bobadilla-Morales, L., Troyo-Sanromán, R., Ramírez-Herrera, M.A., 2006. Cytotoxic effect of curcumin on *Giardia lamblia* trophozoites. Acta Trop. 8, 152–161. https://doi.org/10.1016/j.actatropica.2006.03.005.

Pilla, R., Gaschen, F.P., Barr, J.W., Olson, E., Honneffer, J., Guard, B.C., Blake, A.B., Villanueva, D., Khattab, M.R., AlShawaqfeh, M.K., Lidbury, J.A., Steiner, J.M., Suchodolski, J.S., 2020. Effects of metronidazole on the fecal microbiome and metabolome in healthy dogs. J. Vet. Intern Med 34 (5), 1853–1866. https://doi.org/10.1111/jvim.15871. Epub 2020 Aug 28. PMID: 32856349; PMCID: PMC7517498.

Pintong, A.R., Ruangsittichai, J., Ampawong, S., Thima, K., Sriwichai, P., Komalamisra, N., Popruk, S., 2020. Efficacy of *Ageratum conyzoides* extracts against *Giardia duodenalis* trophozoites: an experimental study. BMC Complement Med. Ther. 20, 63. https://doi.org/10.1186/s12906-020-2860-6.

Ponce-Macotela, M., Rufino-González, Y., González-Maciel, A., Reynoso-Robles, R., Martínez-Gordillo, M.N., 2006. Oregano (*Lippia* spp.) kills *Giardia intestinalis* trophozoites in vitro: antigiardiasic activity and ultrastructural damage. Parasitol. Res. 98, 557–560. https://doi.org/10.1007/s00436-005-0082-y.

Rahman, I.U., Ijaz, F., Afzal, A., Iqbal, A., Ali, N., Khan, S.M., 2016. Contributions to the phytotherapies of digestive disorders: traditional knowledge and cultural drivers of Manoor Valley, Northern Pakistan. J. Ethnopharmacol. 192, 30–52. https://doi.org/10.1016/j.jep.2016.06.049.

Ramsey, J.T., Shropshire, B.C., Nagy, T.R., Chambers, K.D., Li, Y., Korach, K.S., 2020. Essential oils and health. Yale J. Biol. Med. 93, 291–305.

Redouan, F.Z., Yebouk, C., Crisafulli, A., Picone, R.M., Merzouki, A., 2022. Ethnopharmacological preparations used for digestive system disorders in Talassemtane National Park (North of Morocco). Ethnobot. Rese. Appl. 24, 1–25. https://doi.org/10.32859/era.24.2.1-25. Retrieved from. https://ethnobotanyjournal.org/index.php/era/article/view/3839.

Regulation (E.U.) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products. Official Journal of the European Union, L 4, 43–167. ⟨https://eur-lex.europa.eu/eli/reg/2019/6/oj⟩.

Rogawski, E.T., Bartelt, L.A., Platts-Mills, J.A., et al., 2017. Determinants and impact of *Giardia* infection in the first 2 years of life in the MAL-ED birth cohort. J. Pediatr. Infect. Dis. Soc. 6, 153–160. https://doi.org/10.1093/jpids/piw082.

Ryan, U., Caccio, S., 2013. Zoonotic potential of *Giardia*. Int. J. Parasitol. 43, 943–956. https://doi.org/10.1016/j.ijpara.2013.06.001.

Salimikia, I., Yaghoubi, S.E., Khalaf, A.K., Masoori, L., Ghasemian Yadegari, J., Mahmoudvand, H., 2025. Potential activity of *Ferula macrecolea* essential oil for treating *Giardia lamblia* infection through modulating electrolytes and suppressing NF-κB p65 pathway. Front. Pharm. 16, 1542425. https://doi.org/10.3389/fphar.2025.1542425.

Shaverdi, M., Rafiee, Z., Razmjoue, D., et al., 2024. Antibacterial, antioxidant, and anti-giardia properties of the essential oil, hydroalcoholic extract, and green synthesis of the silver nanoparticles of *Salvia mirzayanii* plant. Sci. Rep. 14, 22866. https://doi.org/10.1038/s41598-024-74039-7.

Upcroft, P., Upcroft, J.A., 2001. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin. Microbiol. Rev. 14, 150–164. https://doi.org/10.1128/cmr.14.1.150–164.2001.

Vidal, F., Vidal, J.C., Gadelha, A.P., Lopes, C.S., Coelho, M.G., Monteiro-Leal, L.H., 2007. *Giardia lamblia*: the effects of extracts and fractions from *Mentha x piperita* Lin. (Lamiaceae) on trophozoites. Exp. Parasitol. 115, 25–31. https://doi.org/10.1016/j.exppara.2006.05.001.