Antileishmanial effects, cellular mechanisms, and cytotoxicity of *Elettaria cardamomum* essential oil against *Leishmania major* infection

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ARTICLE HISTORY

Received: 24 April 2023
Revised: 23 May 2023
Accepted: 23 May 2023
Published: 30 June 2023

ABSTRACT

Leishmaniasis is an infectious disease with various clinical manifestations. We studied the therapeutic effects of *Elettaria cardamomum* essential oil (ECEO) against *Leishmania major* infection. In vitro effects of ECEO against *L. major* were examined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and macrophage assays. Nitric oxide (NO) production, infection inhibition in macrophages, and the apoptotic activity of ECEO in treated parasites were also measured. By calculating the 50% cytotoxic concentrations (CC₅₀), we studied the cytotoxic effects of ECEO on human macrophage cells (THP-1). The efficacy of ECEO for improving cutaneous leishmaniasis (CL) lesions in mice (BALB/c) was determined by evaluating the sizes of lesions and the number of amastigotes before and after four weeks of treatment. The effects of ECEO on liver and kidney function in the tested mice were also evaluated. ECEO dose-dependently (p<0.001) improved the viability and the mean number of promastigotes and amastigote forms of *L. tropica*. Four weeks of treatment with ECEO at the doses of 2.5 and 5 mg/kg/day significantly (p<0.001) improved the CL lesions and reduced the number of parasites in the infected mice. ECEO significantly increased NO production, apoptosis induction, and infection rate in parasites. The CC₅₀ value for ECEO and MA was 303.4 µg/mL and 835.2 µg/mL, respectively. In the mice receiving ECEO at the doses of 2.5 and 5 mg/kg/day for 28 days, no significant change was reported between the serum level of liver enzymes and kidney factors when compared with the control group. ECEO displayed promising efficacy in parasite reduction in vitro and in the animal model. ECEO can thus be used as an alternative medicine to treat CL.

Keywords: *Leishmania major*; amastigote; cytotoxicity; herbal medicines; apoptosis.

INTRODUCTION

Leishmaniasis, caused by different species of *Leishmania*, is an infectious disease with various clinical manifestations (Torres-Guerrero et al., 2017). The disease is manifested as cutaneous, mucosal, and visceral leishmaniasis, and >90% of the cases of cutaneous (CL) forms have been reported in Afghanistan, Brazil, Iran, Peru, Iraq, Yemen, and Syria (Mahmoudvand et al., 2011; Alananzi et al., 2016; Alemayehu & Alemayehu, 2017). Although CL is a self-limiting disease, it takes a long time for the lesions to heal. In chemotherapeutics, pentavalent agents, e.g., meglumine antimonate (glucantime, MA) and sodium stibogluconate (pentostam), are broadly used for CL therapy (Nafari et al., 2020). Nevertheless, the use of these medications is restricted due to their side effects, low efficacy, induction of parasitic resistance, and high costs (Albalawi et al., 2021a; AlMohammed et al., 2021). Consequently, it is crucial to discover new drugs that are more effective and less toxic.

The use of plants and plant-derived products has received more attention due to less toxicity, lower cost, and high efficiency (Ullah et al., 2020). Recently, a broad spectrum of herbs plants, e.g., *Zataria* spp., *Pistacia* spp., *Menta* spp., and *Allium* spp. have been studied for their anti-leishmanial effects (Bahmani et al., 2017); however, full approval of the use of medicinal plants to treat CL is hindered by unreliable results of some studies. *Elettaria cardamomum* L. (Zingiberaceae family) is usually used in foods, industry, and folk medicine (Ashokkumar et al., 2020). In traditional medicine, *E. cardamomum* is used as a pain killer, gastrointestinal medicine, and an anti-infective agent (Kumar & Kumari, 2021). Likewise, the modern pharmaceutical uses of *E. cardamomum* are derived from its anti-diabetic, analgesic, inflammation-reducing, and antimicrobial activities (Vutakuri & Somara, 2018; Kumar & Kumari, 2021). Previous studies demonstrated that these pharmacological uses of *E. cardamomum* are principally related to several constituents, e.g., 1,8-cineole, limonene, alpha-terpinol acetate, linalool, and myrcene (Ashokkumar et al., 2021). In the current study, we examined the inhibitory effects and cellular mechanism of *E. cardamomum* essential oil (ECEO) against *Leishmania major* infection.
MATERIALS AND METHODS

Plant collections
Seeds of *E. cardamomum* were purchased from an herbal shop in Shaqra City, Saudi Arabia. After identification by a botanist (Dr. Misfer AlQhatani), a sample was archived at the herbarium of the Shaqra University, Saudi Arabia (No. 25012022).

Preparing essential oil
To extract the essential oil, we used the hydro-distillation procedure by using the Clevenger device (Mahmoudvand et al., 2016a; Alyousif et al., 2021). To perform this, one hundred grams of powdered seeds were placed inside to Clevenger device and lasted for 4 h. The essential oil was then dehydrated by sodium sulfate and kept at 4°C.

Gas chromatography–mass spectrometry (GC-MS)
Chemical composition of ECEO was perfumed based GC-MS according to the conditions described elsewhere by using Hewlett-Packard 6890 (Palo Alto, CA, USA). The obtained constituents were then determined through the evaluation of the obtained mass spectra when compared with the National Institute of Standards and Technology (NIST) mass spectral library (NIST, 2014) and mass spectra reported by Adams (2004).

Cell culture
The human macrophage cell lines (THP-1) were kindly prepared by Faculty of Science and Humanities, Shaqra University, Saudi Arabia, and stored in 75-T cell culture flasks. The medium used to maintain and proliferate these cells was RPMI1640 (Sigma, St. Louis, MO, USA), enhanced with 10% fetal bovine serum (FBS) (Sigma, USA), which was kept at 37°C with 5% CO₂.

Parasite
*L. major* promastigotes (MHOM/TM/82/Lev) were kindly prepared by the Faculty of Science and Humanities, Shaqra University, Saudi Arabia kept in medium containing Novy–MacNeal–Nicolle (NNN) and RPMI1640 medium supplemented with 10% FBS at 25°C (Albalawi et al., 2021b).

Anti-promastigote effects
The anti-leishmaniasis activity of ECEO on cell growth and proliferation of promastigotes was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mahmoudvand et al., 2016b). Logarithmic phase promastigotes in the (2×10⁶/mL) with ECEO (5, 10, 25, 50, 100 µg/mL) were added to 96-wells plates at 24°C for 72 h (the selection of these concentrations was based on the primary experiments and cytotoxicity assay). After this period, the plate containing the parasite and drug was centrifuged, and then MTT powder (0.25 mg/mL) was poured. After four hours of incubation at 24°C, by adding the sulfoxide Dimethyl (DMSO) solution the absorption of the plates was recorded by an ELISA reader (BioteK-ELX800) at 540 nm. Amphotericin B (Gibco, Grand Island, NY, USA) was utilized as the positive control.

Anti-intracellular amastigote effects
First, we transferred macrophage cells (1×10⁵/mL) to each well of the 24-well Lab-Tek with 1 cm² coverslip at 37°C in 5% CO₂ to permit the parasites to invade the macrophages. After 24 h and removing the nonadherent cells, the stationary phase promastigotes (1×10⁵/mL) were transferred to the plates (the ratio of the number of macrophages to *Leishmania* was 1:10). In the next step, ECEO or MA at various concentrations was added to each well containing the *L. major*-infected macrophage for 48 h. Finally, the coverslips were stained with Giemsa solution (10%) and observed under a light microscope. The amastigotes number among 100 macrophages was recorded, and the inhibitory concentration 50 (IC50) for *Leishmania* was determined. MA (Sanofi-Aventis, St Paulo, Brazil) was used as a reference drug in all experiments (Mahmoudvand et al., 2016c).

Infectivity ratio in macrophages
To perform this test, promastigote parasites (1×10⁶/mL) were treated with ECEO (5 µg/mL) for 2 h at 21°C, and they were exposed to macrophage for 4 h. The resulting cells were stained with Giemsa solution, and at least one hundred cells were observed by a light microscope.

Apototic activity of ECEO
ECEO activity on apoptosis of promastigotes was evaluated by assessment of caspase-3 level in promastigotes exposed to ECEO as previously described elsewhere (Albalawi et al., 2021c). The apoptosis was evaluated by measuring the release of substrate-bound PNA (peptide nucleic acid) at 405 nm using a spectrophotometer. Briefly, promastigotes (1×10⁶) were treated with ECEO for 48 h incubation. The cells were centrifuged at 2500 rpm for ten minutes, then, the supernatant (5 µL) was mixed with caspase-3 substrate (10 µL, pNA-DEV-D-AC) and buffer (85 µL) and kept for 120 min at 37°C. The activity was determined by absorbance at 405 nm with an ELISA reader.

ECEO activity on the production of nitric oxide (NO)
Macrophage cells (1×10⁶/mL) were exposed to ECEO at 4.9, 9.8, and 19.6 µg/mL for 48 h. The supernatants of the mixture (10 µL) were moved into a 96-well microplate. In the next step, and after adding the Griess reagent A (50 µL) and B (50 µL) to each well, the OD of wells was measured at 540 nm via an ELISA reader (BioteK-ELX800), and the amount of NO production in THP-1 cells was recorded. The lipopolysaccharide (10 ng/mL) along with IFN-γ (10 U/mL) was utilized as the positive control.

Cytotoxic effects on THP-1 macrophages cells
THP-1 macrophages cells (1×10⁶/mL) in each well were exposed to different concentrations of the ECEO (5, 10, 25, 50, 100 µg/mL) for 48h at 37°C with 5% CO₂ based on the protocol described elsewhere (Mahmoudvand et al., 2017a). The colorimetric MTT analysis was then accomplished as described above. After calculating the 50% cytotoxic concentrations (CC₅₀), the selectivity index (SI) ratio was determined by calculation of CC₅₀ for macrophage/IC₅₀ for amastigotes.

In vivo efficacy of ECEO against CL in mice

Animals
Thirty-two male BALB/c mice aged 40 to 60 days were used for in vivo assays and separated into four groups having eight mice. Mice were kept in a room under well-ordered temperature (24 ± 1°C), lighting, and humidity of 40–70%. Mice also take a regular diet and water ad libitum.

Ethics statement
This work is permitted by the Ethics Committee at Almaarefa University, Saudi Arabia (IRB06-25012022-09).

Animal model of CL
Through the subcutaneous injection of the stationary phase promastigotes (0.1 mL, 1×10⁷ cells) into the mice’s tail.

CL treatment by ECEO
Forty days post-infection, when lesions were formed and observed, animals were topically treated with ECEO (2.5 and 5 mg/kg/day) as a topical ointment for 28 days. Furthermore, mice in the negative control groups received normal saline and MA (30 mg/kg/day), respectively.
Effect of ECEO on CL in mice
To study the effect of ECEO on CL in mice, changes in lesion sizes of CL were monitored at the beginning of therapy, 2nd week, and 4th week of drugs employing a Vernier caliper (Mahmoudvand et al., 2016b). Parasite loads were determined in the same timetables followed by providing the lesions smear, Giemsa staining, and assessment by a light microscope.

Effect of ECEO on liver and kidney function
To determine the effects of ECEO on liver and kidney function in tested mice, the blood samples from the all tested mice were collected by cardiac puncture. After centrifuging the samples at 3500 rpm for 15 min, the obtained sera were stored at –20°C until testing (Mahmoudvand et al., 2017b; Keyhani et al., 2020). Finally, using the commercial diagnostic kits (Roche, Germany), the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cr), and blood urea nitrogen (BUN) were calculated.

Statistical analysis
SPSS 25.0 version software was used for data analysis. All experiments were accomplished in triplicate. A one-way analysis of variance (ANOVA) test was utilized for the assessment of results between groups. If significant, the post hoc Dunnett test was used to determine the effectiveness of the control drug and essential oil. IC\textsubscript{50} and CC\textsubscript{50} values were calculated using the Probit test. p<0.05 was presented as a significant difference.

RESULTS

Gas chromatography–mass spectrometry analysis
The results showed that ECEO yielded 3.74% (v/w). The GC/MS results revealed that 20 compositions were found, representing 99.3% of the essential oil (Table 1). The highest amounts were observed for monoterpenes compositions, e.g., 1,8-cineole (42.3%), α-terpinyl acetate (18.2%), and α-pinene (13.7%), respectively.

Effect on promastigote stages
After 72 h of exposure to promastigotes, with increasing the ECEO concentration, the viability of promastigotes markedly decreased (p<0.001). The IC\textsubscript{50} value for ECEO and Amb was 19.6 and 1.36 µg/mL, respectively (Table 2).

Effect on amastigote stages
The in vitro assay of the effects of ECEO on amastigote stages of *L. major* revealed that after treatment of the infected macrophages with ECEO, the number of amastigotes noticeably dropped (p<0.001) dose-dependently. The IC\textsubscript{50} value for ECEO and MA was 29.8 µg/mL and 44.3 µg/mL, respectively (Table 2).

Infecitivity ratio in macrophages
Based on the current experimental investigation, exposure to promastigotes with ECEO significantly reduced (p<0.001) the infection rate. The infection rate in the non-treated macrophage cells and the cells treated with ECEO (5 µg/mL) was 83.6% and 26.3%, respectively (Table 3).

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>RI*</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>α-pinene</td>
<td>1022</td>
<td>3.7</td>
</tr>
<tr>
<td>2.</td>
<td>α-thujene</td>
<td>1022</td>
<td>2.6</td>
</tr>
<tr>
<td>3.</td>
<td>Camphene</td>
<td>1069</td>
<td>0.4</td>
</tr>
<tr>
<td>4.</td>
<td>Sabinene</td>
<td>1136</td>
<td>2.1</td>
</tr>
<tr>
<td>5.</td>
<td>Myrcene</td>
<td>1189</td>
<td>2.4</td>
</tr>
<tr>
<td>6.</td>
<td>Geraniol</td>
<td>1207</td>
<td>0.8</td>
</tr>
<tr>
<td>7.</td>
<td>α-terpinyl acetate</td>
<td>1214</td>
<td>18.2</td>
</tr>
<tr>
<td>8.</td>
<td>1,8-cineole</td>
<td>1228</td>
<td>38.3</td>
</tr>
<tr>
<td>9.</td>
<td>γ-terpinen</td>
<td>1265</td>
<td>1.7</td>
</tr>
<tr>
<td>10.</td>
<td>p-Cymene</td>
<td>1271</td>
<td>1.1</td>
</tr>
<tr>
<td>11.</td>
<td>Octanal</td>
<td>1318</td>
<td>0.4</td>
</tr>
<tr>
<td>12.</td>
<td>trans-Sabinene Hydrate</td>
<td>1465</td>
<td>0.6</td>
</tr>
<tr>
<td>13.</td>
<td>Linalyl acetate</td>
<td>1533</td>
<td>2.4</td>
</tr>
<tr>
<td>14.</td>
<td>Linalool</td>
<td>1543</td>
<td>3.6</td>
</tr>
<tr>
<td>15.</td>
<td>cis-4-Decenal</td>
<td>1556</td>
<td>0.9</td>
</tr>
<tr>
<td>16.</td>
<td>Borneyl acetate</td>
<td>1611</td>
<td>0.8</td>
</tr>
<tr>
<td>17.</td>
<td>Carvone</td>
<td>1612</td>
<td>2.2</td>
</tr>
<tr>
<td>18.</td>
<td>Pinocarvone</td>
<td>1617</td>
<td>0.7</td>
</tr>
<tr>
<td>19.</td>
<td>4-terpinen-4-ol</td>
<td>1626</td>
<td>1.8</td>
</tr>
<tr>
<td>20.</td>
<td>trans-Pinocarveol</td>
<td>1681</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 2. The IC\textsubscript{50} and CC\textsubscript{50} values (µg/mL) determined for the *E. cardamomum* essential oil (ECEO), compared with the meglumine antimoniate (MA), and amphotericin B (Amb), as well as the selectivity index (SI) against intramacrophage amastigote forms of *Leishmania major*. The findings were indicated as mean ± standard deviation (n=3)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Promastigote IC\textsubscript{50} (µg/mL)</th>
<th>Amastigote IC\textsubscript{50} (µg/mL)</th>
<th>CC\textsubscript{50} (µg/mL) of the J774-A1 Cells</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECEO</td>
<td>19.6 ± 1.68</td>
<td>29.8 ± 2.65</td>
<td>303.4 ± 12.3</td>
<td>10.2</td>
</tr>
<tr>
<td>MA</td>
<td>–</td>
<td>44.3 ± 3.012</td>
<td>835.2 ± 9.2</td>
<td>18.8</td>
</tr>
<tr>
<td>Amb</td>
<td>1.36 ± 0.045</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3. Effect of *Elettaria cardamomum* essential oil (ECEO) on inhibition of infection in macrophages in comparison with the meglumine antimoniate (MA). Mean ± SD (n=3)

<table>
<thead>
<tr>
<th>Promastigotes</th>
<th>% of infected macrophages</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td>83.6 ± 4.78</td>
<td>–</td>
</tr>
<tr>
<td>ECEO (5 µg/mL)</td>
<td>26.3 ± 2.36</td>
<td>68.5***</td>
</tr>
<tr>
<td>MA</td>
<td>35.3 ± 3.66</td>
<td>57.4</td>
</tr>
</tbody>
</table>

*** p < 0.001 difference was statistically significant compared with the negative control.
Effect on apoptosis in *Leishmania* parasites

The colorimetric protease assay results exhibited that ECEO, mainly at 9.8 and 19.6 µg/mL, significantly prompted the caspase-3 activity by 21.5 and 36.7%, respectively (Figure 1).

Effect on NO production

We measured the released NO in macrophages treated with ECEO through the Griess reaction for nitrates. Table 4 exhibits the effect of ECEO on the production of NO in THP-1 macrophage cells. NO production was provoked, and a significant (p<0.001) production was reported at 9.8 and 19.6 µg/mL compared to the control group.

Cytotoxic effects on THP-1 macrophage cells

The CC_{50} value for ECEO and MA was 303.4 µg/mL and 835.2 µg/mL, respectively. Consequently, the calculated SI of >10 for ECEO and MA confirmed their specificity to amastigotes, as well as their low toxicity for macrophages (Table 2).

*In vivo* effect of ECEO on CL in mice

After ECEO therapy at 2.5 and 5 mg/kg/day, the mean diameter of the CL lesions considerably declined, such that at 5 mg/kg, the CL lesions disappeared, and complete healing occurred (Figure 2A). After the MA therapy, the CL size considerably decreased by 9.4 mm. Microscopic examinations also exhibited that after four weeks of treatment with ECEO at the doses of 2.5 and 5 mg/kg/day, the number of parasites in the infected mice markedly declined in comparison with the control mice (Figure 2B).

Effect of ECEO on liver and kidney function

Concerning the possible toxicity for liver and kidney function, the biochemical findings revealed that no significant change was reported in the serum level of liver enzymes and kidney factors of the mice receiving ECEO topically at the doses of 2.5 and 5 mg/kg/day for 28 days compared with the control group (P > 0.05) (Figure 3).

![Figure 1. The effect of *Elettaria cardamomum* essential oil (ECEO) on Caspase-3-like activity of *L. major* promastigotes by the colorimetric protease methods. The findings are indicated as mean ± standard deviation. *** p < 0.001 shows the difference was statistically significant in comparison with control.](image)

![Figure 2. (A) Effect of various doses of *Elettaria cardamomum* essential oil (ECEO) on the lesions size in BALB/c mice infected by *Leishmania major*. The findings are indicated as mean ± standard deviation. *** p < 0.001 shows the difference was statistically significant in comparison with control. (n=8); (B) effect of various doses of *Elettaria cardamomum* essential oil (ECEO) on the mean number of parasites (parasite load) in BALB/c mice infected by *L. major*. The findings are indicated as mean ± standard deviation. *** p < 0.001 shows the difference was statistically significant in comparison with control. (n=8).](image)

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>NO production (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>6.56 ± 0.58</td>
</tr>
<tr>
<td>9.8</td>
<td>16.3 ± 1.15***</td>
</tr>
<tr>
<td>19.6</td>
<td>21.3 ± 1.62***</td>
</tr>
<tr>
<td>Non-treated</td>
<td>4.71 ± 0.26</td>
</tr>
<tr>
<td>IFN-γ+LPS</td>
<td>33.6 ± 3.32</td>
</tr>
</tbody>
</table>

*** p < 0.001 difference was statistically significant compared with the negative control.

Table 4. The effect of *Elettaria cardamomum* essential oil (ECEO) on nitric oxide (NO) production in human macrophage cell line (THP-1) in comparison with the positive (IFN-γ+LPS) and negative controls (non-treated). The findings are indicated as mean ± standard deviation (n=3)
DISCUSSION

With the increasing prevalence of CL, treatment approaches, especially chemotherapy with pentavalent antimony compounds, have received considerable attention due to the lack of effective vaccines. However, the use of these drugs is limited and associated with side effects (Rojas et al., 2006). Therefore, it is essential to use alternative compounds such as natural products and native medicinal plants of the region whose therapeutic effects and low toxicity have been proven. Herein, we studied the inhibitory effects and cellular mechanisms of ECEO against *L. major* infection.

Our results showed that after 72 h of incubation of promastigotes with ECEO, the viability of promastigotes significantly decreased (*p*<0.001). The *in vitro* assay of the effects of ECEO on amastigote stages of *L. major* revealed that after treatment of macrophages infected with amastigotes with ECEO, the number of amastigotes dose-dependently declined (*p*<0.001). On the other hand, after four weeks of ECEO therapy at the doses of 2.5 and 5 mg/kg/day, the mean diameter of the lesions and their parasite load considerably decreased, such that at the dose of 5 mg/kg, the CL lesions disappeared and complete healing occurred.

Although the antileishmanial activity of several medicinal herbs, e.g., *Z. multiflora*, *Z. spina-christi*, *M. communis*, *P. vera*, *T. africana*, and *P. tomentosa* has been studied (Mahmoudvand et al., 2015; Albalawi et al., 2021d), complete acceptance of their use to treat CL is hindered by unreliable results of studies with inadequate power. The antimicrobial effects of cardamom against pathogenic parasites have been evaluated in a few studies; for example, in a survey conducted by Farrag et al. (2021), the results revealed the *in vitro* and in vivo effects of *E. cardamomum* at concentrations of 1-2.5 mg/mL on the parasite load, infectivity rate, and improving the signs in male Wistar rats with *Trypanosoma evansi*. Almohammed et al. (2022) also reported that *E. cardamomum* at the concentration of 200 µl/mL completely killed *Echinococcus granulosus* protoscolices after 10 min of exposure. Furthermore, antimicrobial activities of *E. cardamomum* against various bacterial (e.g., *Escherichia coli*, *Staphylococcus* spp., *Salmonella* spp. *Bacillus cereus*) and fungal species (e.g., *Aspergillus* spp., and *Candida* spp.) have been examined by several studies (Noshad et al., 2019).

The GC/MS results showed that 20 compositions were found, representing 99.3% of the essential oil (Table 1). The highest amounts were observed for monoterpenes compositions, e.g., 1,8-cineole (42.3%), α-terpinyl acetate (18.2%), and α-pinene (13.7%), respectively. Among the secondary metabolites present in essential oils, monoterpenes compounds as primary metabolites are widely considered for planning, discovering, and developing new pharmacological agents (Wojtunik-Kulesza et al., 2019). In terms of antimicrobial activities of monoterpenes compounds, various surveys reported that some of these compounds, e.g., α-pinene, 1,8-cineole, α-terpinyl acetate display potent antibacterial, antifungal, antiparasitic, and antiviral effects (Rodrigues Goulart et al., 2004; Zielinska-Bajet & Feder-Kubis, 2020). As the antimicrobial action of monoterpene compounds is not understood, several studies have reported that these compounds revealed their mechanism of action by interrupting the cell membrane, inhibiting oxygen consumption by microorganisms, and inhibiting the effect of virulence of factors (Boy et al., 2018). Recently, Moo et al. (2021) have demonstrated that 1,8-cineole displays its antimicrobial action by affecting some cellular mechanisms, e.g., causing the leakage of proteins and nucleic acids, inducing oxidative stress, producing reactive oxygen species (ROS), disrupting cell membrane, and losing intracellular materials. Studies also showed that other compounds of ECEO, e.g., α-pinene and α-terpinyl acetate, exhibit their mechanism of action through several cellular mechanisms,
CONCLUSION

ECEO displayed promising results in parasite reduction in both in vitro and in vivo assays. ECEO may be used as a new drug to treat CL caused by L. major. Nevertheless, additional investigations are mandatory to report its exact mechanisms of action and efficacy in clinical trials. We confirmed that the use of native herbs and their active ingredients in regions such as Saudi Arabia, where leishmaniasis is endemic, can help the invention and discovery of effective and safe herbal-based drugs for CL treatment.

ACKNOWLEDGEMENTS

The authors would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

Conflict of interest statement

The author declares that they have no competing interests.

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