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Chemical composition, antileishmanial, and cytotoxic effects *Ferula macrecolea* essential oil against *Leishmania tropica*

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ABSTRACT

Background: The current study was aimed to evaluate the chemical composition, as well as antileishmanial and cytotoxic effects of the essential oil of *Ferula macrecolea* and its main compound, terpinolene, against promastigotes and amastigotes of *Leishmania tropica.*

Methods: The chemical composition of the essential oil was analyzed by a gas chromatograph connected to a mass spectrometer (GC/MS). The MTT (3-(4.5-dimethylthiazol-2-yl) − 2.5-diphenyl tetrazolium bromide) assay was used to study the effects of the essential oil and terpinolene against promastigotes while the macrophage model was used for evaluating the effect of *F. macrecolea* essential oil against amastigotes of *L. tropica* as well as assessing cytotoxicity. The Griess reaction assay was employed to study the nitric oxide (NO) produced by treating macrophage cells with the essential oil and terpinolene. Furthermore, the effect of the essential oil and terpinolene on plasma membrane permeability and inhibition of infection in macrophages was evaluated.

Results: The main compounds were terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%), respectively. The 50% inhibitory concentrations (IC_{50}) of the essential oil, terpinolene, and glucantime against promastigotes were 27.6, 11.6, and 32.8 μg/mL, respectively; however, their IC_{50} values against amastigotes were 42.3, 19.6, and 56.9 μg/mL, respectively. The 50% cytotoxic concentrations of the essential oil, terpinolene, and glucantime were 471.3, 207.3, and 1165.3 μg/mL, respectively. The production of NO in macrophage cells after treatment with the essential oil and terpinolene was increased in a dose-dependent manner ($p < 0.001$). The results revealed that by increasing the concentration of the essential oil and terpinolene, the permeability of the parasites' plasma membrane was significantly changed ($p < 0.001$). The pre-incubation of *Leishmania* parasites with *F. macrecolea* essential oil and terpinolene significantly declined the rate of cell infection by 74.8% and 79.4%, respectively ($p < 0.001$).

Conclusion: The results of the present study indicated that *F. macrecolea* essential oil, especially its main compound, i.e., terpinolene, has a potent antiparasitic effect on the promastigote and amastigote stages of L. *tropica.* Considering the advantages of medicinal plant products over their chemical counterparts, it is suggested that in the continuation of this study, the effect of *F. macrecolea* essential oil, especially terpinolene, on laboratory animals, and in case of high efficiency, in humans be evaluated.

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1. Introduction

Leishmaniasis is a spectrum of diseases caused by *Leishmania* intracellular protozoa. It is estimated that there are about 12 million cases of cutaneous leishmaniasis (CL) worldwide, and 350 million people are currently at risk in nearly 90 countries (Monzote, 2009). Leishmaniasis causes a wide range of human infections, from spontaneously healing skin lesions to diffuse mucosal skin forms and sometimes fatal visceral lesions (kalazar) (Torres-Guerrero et al., 2017). According to the World Health Organization (WHO), Iran is among the 10 countries where >70–75% of global cases of CL are reported. Iran ranks first in the Middle East in terms of CL and fourth in terms of visceral leishmaniasis (Shirzadi et al., 2015).

At present, various treatments have been used to treat CL, which generally include physical or surgical treatment and medication (Arana et al., 2001). Physical and surgical treatments include cryotherapy, local heat, curettage, and argon laser (Arana et al., 2001). The most important treatments for leishmaniasis today are the pentavalent antimony compounds, which include sodium stibogluconate (pentostam) and meglumine antimonate (glucantime) (Brito et al., 2017). Due to the numerous side effects of the drug, efforts are underway to find a new drug that can heal the wound faster, has the fewest side effects, and after healing, does not leave a scar (Oliveira et al., 2011; Santos et al., 2008). Therefore, the study of medicinal plants, especially native plants of the region, to find a suitable drug against leishmaniasis is of great importance. Natural compounds and plant-derived substances are widely used against pathogenic microorganisms (Rocha et al., 2005).

Ferula plants with >150 different species are among the most widely used plants in traditional medicine in the world (Yaqoob and Nawchoo, 2016). *Ferula macrecolea* Boiss is one of the main plants in this genus with various therapeutic properties, e.g., analgesic, anti-inflammatory, antihypertensive, antibacterial, anti-parasitic, antiviral, antifungal, and insecticidal effects, which is also involved in treating cardiovascular and gastrointestinal diseases in traditional and modern medicine (Moreira et al., 2014; Salehi et al., 2019). Investigations have reported that terpenoid combinations (e.g., terpinolene, α-pinene, and myrcene) are the main compounds in the essential oils of *Ferula* spp. (Sahebkar and Iranshahi, 2011). The current study was aimed to evaluate the chemical composition, antileishmanial effects, and cellular mechanisms of the essential oil extracted from *F. macrecolea* against promastigotes and amastigotes of *L. tropica.*

2. Materials and methods

2.1. Ethics

The protocol of this experimental study is accepted by ethical committee of Lorestan University of Medical Sciences, Khorramabad, Iran (IR.LUMS.REC.1400.202).

2.2. Plant materials

In this study, the aerial parts of the plant were collected from the western regions of Islamabad in Kermanshah province in April 2021 and after confirming and identifying by a botanist the scientific name, an herbarium sample (No. 1400.2276) was prepared and deposited in herbarium of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran). Plants materials were dried and powdered and stored in dark containers.

2.3. Isolation of essential oil

Water distillation method was used to extract the essential oil by Clevenger device. The time to extract the essential oil was 4 h; after extracting, the essential oil was separated from the surface of the water and dried with sodium sulfate. Until the chemical and antileishmanial tests were performed, the extracted essential oil was kept at refrigerator temperature in clean containers wrapped in aluminum foil (Mahmoudvand et al., 2017a; Mahmoudvand et al., 2017b).

2.4. Gas chromatography–mass spectrometry

After preparing the essential oil, its chemical composition was analyzed by a gas chromatograph connected to a mass spectrometer (GC/MS). The HP6890-Packard-Hewlett GC was equipped with a column 30 m long and 25 mm in diameter and layer thickness The inside was 0.25 μm of 5MS-HP type. The temperature program of this column was as follows: initial temperature 50 °C and stop at this temperature for 5 min, then heat gradient up to 250 °C, with a gradual increase of 5 °Celsius per minute and keep the column at 250 °C for 20 min. The helium gas velocity was 1 mL / min. The mass spectrometer used was Model 5975 Agilent with ionization voltage of 70 eV, EI ionization method and ionization source temperature of 220 °C. The compounds of essential oil were identified through assessment of the retention index and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases (Adams, 2004a; Albalawi et al., 2021a; NIST, N, 2014).

2.5. Preparation of the terpinolene

Terpinolene ($C_{10}H_{16}$), as the main compounds of *F. macrecolea* essential oil prepared from Sigma-Aldrich, (St. Louis, MO, USA), was

dissolved in the dimethyl sulfoxide (DMSO).

2.6. Parasite

The standard strain of *L. tropica* (MHOM/AF/88/KK27) was obtained from Pasteur Institute, Tehran, Iran. Standard strains promastigotes were cultured in a 25 mL flask containing 1640 RPMI medium containing 10% bovine fetal serum (FBS), penicillin antibiotics (100 mL/IU) and streptomycin (100 mL/IU) at 24 °C was maintained (Albalawi et al., 2021a).

2.7. Cell culture

The macrophage cells used in this work (J774-A1 cell) were prepared from Pasteur Institute, Tehran, Iran. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) developed with 10% FBS at 37 °C with 5% CO_2 (Albalawi et al., 2021a).

2.8. Effect on promastigote forms of L. **tropica**

In summary, 1×10^6 promastigotes were added to each 96-well plate, and concentrations of 6.25–100 μg/mL of essential oil and its main compound, terpinolene were added to each well containing the parasite and incubated at 24 °C for 48 h. After the desired time, the supernatant was discarded and then 10 μL of MTT ([3-(4.5-dimethylthiazol-2-yl) − 2.5-diphenyl tetrazolium bromide)]) solution (5 mg/mL, with the final concentration in well of 0.5 mg/mL) was added to each well and stored for 4 h under 5% CO_2 at 37 °C. Furamazan crystals were then dissolved by adding 100 μL of dimethylsulfoxide and the absorbance from each well (at 570 nm) obtained using an ELISA plate reader, Oraganon Teknika (Albalawi et al., 2021a). Glucantime was also considered as the reference drug in this experiment.

2.9. Effect on amastigote forms of L. **tropica**

To do this, 1×10^5/mL of macrophage cells to the plated in 24- well plate with 1 cm^2 cover slips positioned on floor at 37 °C in 5% CO_2 for 24 h. After removing the non-adherent cells, 100 μL of *L. tropica* promastigotes (1×10^6/mL) in stationary phase were added to the wells (at ratio of 10:1) and then were incubated at 37 °C in 5% CO_2 for 24 h. Different concentrations of essential, and its main compound, terpinolene, and glucantime were distinctly added to each well comprising infected macrophage cells for 48 h. As a final point, slides were fixed in absolute methanol, stained with Giemsa dye, and studied under light microscopy. Number of amastigotes forms in 100 macrophages were recorded and the 50% inhibitory concentrations (IC_{50}) were determined. The examinations were carried out in triplicate and the findings were indicated as mean ± standard deviation (Mahmoudvand et al., 2015a).

2.10. Inhibition of macrophage infection by the parasite

The infectivity is one of the key pathogenic and biological criteria of *Leishmania* spp., that the new drugs can prevent the growth of the parasite and finally the occurrence of clinical symptoms by inhibiting the infection in macrophages (Albalawi et al., 2021b). Here, we investigated the effect of essential oil on the inhibition of macrophage infection by the parasites. To do this, promastigotes were initially (before infection of macrophages) exposed with essential oil and its main compound, terpinolene (5 μg/mL) with medium for 4 h at 24 °C. Afterward, pre-treated *L. tropica* promastigotes (1×10^6/mL) in stationary phase were added to the wells containing macrophage cells (1×10^5/mL) at ratio of 10:1 and then were incubated at 37 °C in 5% CO_2 for 24 h. Next, slides were prepared from cells and after staining with Giemsa stain, they were studied under a light microscope via checking 100 cells (Albalawi et al., 2021b).

2.11. Effect on permeability of Leishmania plasma membrane

In order to investigate the effect of essential oil and its main compound, terpinolene on permeability of *Leishmania* plasma membrane, after treating the promastigotes (1×10^6 cells/mL) with of essential oil at the doses of ¼, 1/3, and ½ IC_{50} for 4 h at 24 °C, the mixture was then incubated with SYTOX® green stain for 15 min. Finally, the absorbance (optical density, OD) of reaction was read using a microplate reader every 60 min for 4 h. Parasites treated with Triton X-100 (2.5%) and those with no drug were positive and negative control, respectively (Albalawi et al., 2021b).

2.12. Determining the nitric oxide (NO) production

Griess reaction assay was used to study NO produced by treating macrophage cells with essential oil and its main compound, terpinolene. In this method, after exposing the macrophage cells (1×10^5/mL) with various concentration of essential oil (1/4 IC_{50}, ½ IC_{50}, and IC_{50}) for 48 h, in a 96-well plate the supernatant of reaction (20 μL) was mixed with the nitrite assay buffer (80 μL), Griess reagent A (10 μL, Sigma-Aldrich) and B (10 μL) the amount of NO was recorded at 540 nm in an ELISA reader (BioTek-ELX800) (Albalawi et al., 2021b). Standard curve (a serial dilution from 1 to 100 μM nitrite) was also prepared from 1 mM nitrite standard solution. The cells treated with the combination of lipopolysaccharide (LPS, 10 ng/mL) along with IFN-γ (10 U/mL) were considered as the positive control.

2.13. Cytotoxic effects of J774-A1 macrophage cells

Macrophage cells (1×10^5/mL) were added to each 96-well plate, and various concentrations of essential oil and its main compound, terpinolene were separately added to each well containing the parasite and incubated at 24 °C for 48 and 72 h. Then, similar to promastigote assay, by MTT assay, the 50% cytotoxic concentrations (CC_{50} values) was evaluated absorbing light from each at a wavelength of 570 nm by an ELISA reader. Also, to study cytotoxicity and efficacy of essential oil, the selectivity index (SI) was measured based on the equation CC_{50} /IC_{50} for amastigotes (Mahmoudvand et al., 2015b).

2.14. Statistical analysis

The results of this study were analyzed using SPSS software version 26.0 and one-way analysis of variance (ANOVA). Significance level was considered $p < 0.05$. All tests were repeated three times.

3. Results

3.1. GC/MS analysis

Based on the peaks obtained in the GC/MS analysis, out of the 18 compounds identified (98.99%), the main components were terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%), respectively (Table 1).

3.2. Effect on the promastigote forms of L. *tropica*

The results of the MTT test showed that the main compound of the essential oil, terpinolene, had potent antileishmanial effects on *L. tropica* promastigotes by increasing the concentration and the concentration-dependent response ($p < 0.001$). The IC_{50} calculated based on the OD obtained for the essential oil, terpinolene, and glucantime was 27.6 ± 3.14, 11.6 ± 1.52, and 32.8 ± 3.61 μg/mL, respectively (Table 2). Among the tested drugs, terpinolene showed the better ($p < 0.01$) antileishmanial effects on L. *tropica* promastigotes in comparison with glucantime.

3.3. Effect on the intracellular amastigote forms of L. *tropica*

The results of the macrophage model revealed that the essential oil and its main compound, terpinolene, had promising antileishmanial effects on *L. tropica* intracellular amastigote by increasing the concentration such that the mean number of intracellular amastigote significantly declined in a concentration-dependent response ($p < 0.001$). The IC_{50} values calculated for the essential oil, terpinolene, and glucantime were 42.3 ± 4.32, 19.6 ± 2.05, and 56.9 ± 5.12 μg/mL, respectively (Table 2). Based on the obtained results, terpinolene showed the better ($p < 0.01$) antileishmanial effects on *L. tropica* intracellular amastigotes in comparison with glucantime.

Table 1
Chemical composition of *Ferula macrecolea* essential oil by GC/MS analysis.

No.	Composition	RI[a]	RI[b]	Percent (%)
1.	α-Thujene	926	928	3.92
2.	α-pinene	936	940	0.11
3.	p-cymene	1020	1026	0.23
4.	β-phellandrene	1028	1030	1.31
5.	Benzeneacetaldehyde	1032	1043	1.35
6.	Limonene	1036	1030	0.25
7.	Terpinolene	1094	1097	77.72
8.	n-Nonanal	1102	1108	4.47
9.	Linalool	1109	1104	4.35
10.	α-campholenal	1125	1127	0.32
11.	Allo-ocimene	1128	1126	0.42
12.	Geijerene	1146	1150	0.58
13.	Camphor	1148	1149	0.24
14.	Terpinen-4-ol	1186	1177	0.29
15.	Myrtenal	1196	1192	1.22
16.	di-sec-butyl disulfide	1212	1220	0.17
17.	Piperitone	1252	1253	0.1
18.	Carvacrol	1276	1277	1.94
	Total			98.99

a: the obtained retention index; b: retention index in literature.

Table 2
Antileishmanial and cytotoxicity effects of *Ferula macrecolea* essential oil and its main compound, terpinolene by measuring the 50% inhibitory concentrations (IC_{50}) and the 50% cytotoxic concentrations (CC_{50}) values (μg/mL) and selectivity index (SI) against. Mean ± standard deviation. ($n = 3$).

Drug	Promastigote IC_{50} (μg/mL)	Amastigote IC_{50} (μg/mL)	CC_{50} (μg/mL) of the J774-A1 Cells	SI
Essential oil	27.6 ± 3.14	42.3 ± 4.32	473.3 ± 7.83	11.2
Terpinolene	11.6 ± 1.52*	19.6 ± 2.05*	207.3 ± 6.54	10.6
Glucantime	32.8 ± 3.61	56.9 ± 5.12	1165.3 ± 13.2	20.5

* $P < 0.001$ significant difference compared with Glucantime.

3.4. Inhibition of macrophage infection by the parasite

The results of microscopic examinations showed that the percentage of macrophages infected by non-treated promastigotes was 69.8 ± 3.14; however, the percentages of infected macrophages after treatment of promastigotes with the essential oil and its main compound, terpinolene, were 18.61 ± 1.51 and 14.34 ± 2.15, respectively. This finding indicated that the pre-incubation of *Leishmania* parasites with *F. macrecolea* essential oil and terpinolene significantly declined the rate of cell infection by 74.8% and 79.4%, respectively, when compared with non-treated parasites ($p < 0.001$).

3.5. Effect on the permeability of Leishmania plasma membrane

The acquired OD of relative fluorescent units revealed that by increasing the concentration of the essential oil and terpinolene, the permeability of parasites changed significantly ($p < 0.001$) compared to the control group in a dose-dependent manner (Fig. 1).

3.6. Determining the NO production

The Griess reaction assay was used to study the NO produced by treating macrophage cells with the essential oil and terpinolene.

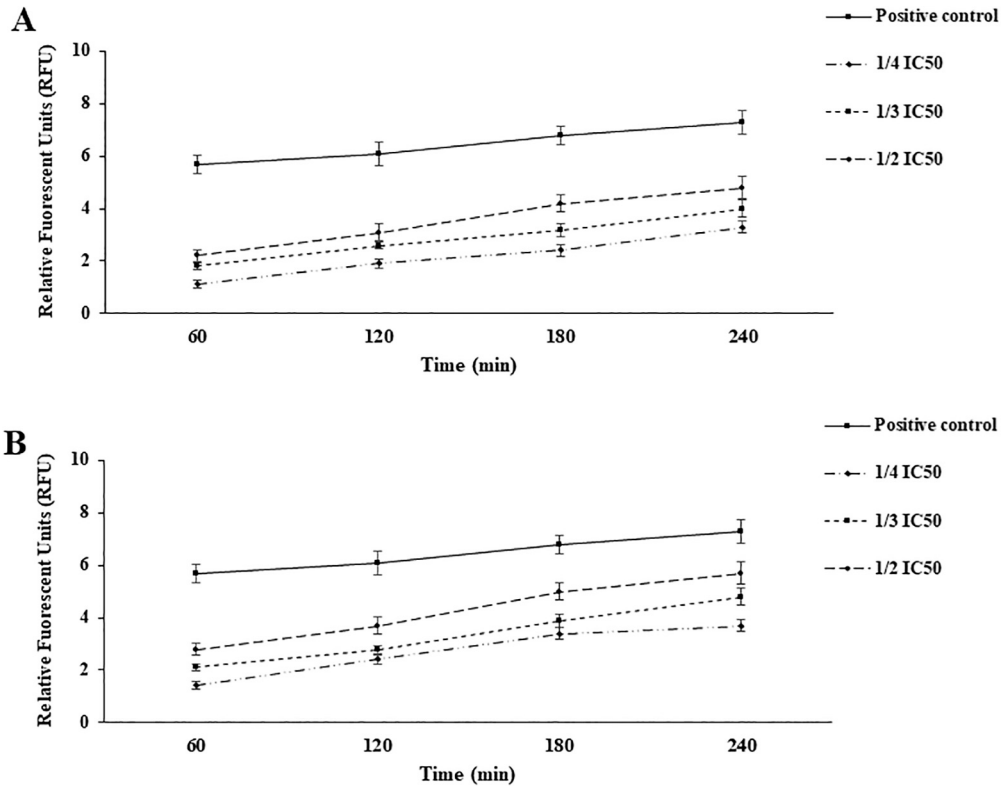

Fig. 1. Effect of various doses of *Ferula macrecolea* essential oil (A) and its main compound, terpinolene (B) on permeability of *Leishmania* plasma membrane by SYTOX® green assay. Mean ± standard deviation (n = 3). IC_{50}: The 50% inhibitory concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Based on the results of the Griess reaction assay, the amount of NO production in macrophage cells after treatment with the essential oil was increased especially at the concentration of ½ IC_{50} and IC_{50} ($p < 0.001$) when compared to the control group (Table 3). The results also revealed that the amount of NO production in macrophage cells treated with terpinolene was significantly elevated ($p < 0.001$) when compared with the control group.

3.7. Cytotoxic effects of J774-A1 macrophage cells

Based on the OD obtained in the MTT assay, the CC_{50} value for the essential oil, terpinolene, and glucantime was 471.3, 207.3, and 1165.3 μg/mL, respectively. Accordingly, the calculated SI of >10 for the essential oil and glucantime indicated their specificity to amastigotes with minimum toxicity on macrophage cells (Table 1).

4. Discussion

Due to the increase in drug resistance and the occurrence of side effects after the use of chemical drugs, researchers have focused on finding natural drugs and alternatives for the treatment of diseases. CL is one of the diseases that is very difficult and time-consuming to treat (AlMohammed et al., 2021). Essential oils, extracts, and other compounds derived from plants are valuable sources for finding anti-leishmaniasis drugs (Rocha et al., 2005). In this study, the anti-leishmanial effects and several cellular mechanisms of the essential oil extracted from *F. macrecolea* against promastigotes and amastigotes of *L. tropica* are evaluated.

Our findings revealed that the essential oil had stronger antileishmanial effects on L. *tropica* promastigotes by increasing the concentration and dose dependence ($p < 0.001$). The IC_{50} calculated based on the OD obtained for the essential oil, terpinolene, and glucantime was 27.6, 11.6, and 32.8 μg/mL, respectively. We also found that the essential oil showed higher antileishmanial effects on L. *tropica* amastigotes by increasing the concentration such that the mean number of amastigotes significantly declined in a dose-dependent manner ($p < 0.001$). The IC_{50} calculated for the essential oil, terpinolene, and glucantime was 42.3, 19.6, and 56.9 μg/mL, respectively. These findings demonstrated that terpinolene had significantly higher anti-leishmaniasis effects on L. *tropica* promastigotes and amastigotes than glucantime and *F. macrecolea* essential oil.

The antimicrobial activities of *Ferula* spp. against a wide range of pathogenic bacteria (e.g., *Bacillus* spp., *Staphylococcus* spp., *Enterococcus faecalis, Salmonella* spp., and *Pseudomonas aeruginosa*) and fungi (e.g., *Candida* spp., *Trichophyton* spp., and *Aspergillus* spp.) have been proven in several studies (Eckert et al., 2001; Boghrati and Iranshahi, 2019; Asili et al., 2009; Maggi et al., 2009; Iranshahi et al., 2008; Ghasemi et al., 2005; Rahman et al., 2008). Few studies have investigated the antiparasitic effects of *Ferula* spp. For example, in a study conducted by Iranshahi et al. (2007), the acetone extract obtained from the root of *F. szowitsiana* had relevant leishmanicidal activity on *L. major* promastigotes ($IC_{50} = 11.8$ μg/mL) (Iranshahi et al., 2007). Esmaeili et al. (2009) have reported the potent antiparasitic effects of the methanolic extract of *F. oopoda* against the *Plasmodium falciparum* K1 ($IC_{50} = 26.6$ μg/mL) and 3D7 ($IC_{50} = 24.9$ μg/mL) (Esmaeili et al., 2009). Moreover, in a study carried out by Khanmohammadi et al. (2014), the methanol extract of *F. szowitsiana* displayed potent antiparasitic effects against trophozoites of *Trichomonas vaginalis* with an IC_{50} value of 0.360 mg/mL (Khanmohammadi et al., 2014). Recently, Alyousif et al. (2021) reported that *F. macrecolea* essential oil displayed effective in vitro and ex vivo protoscolicidal activity against *Echinococcus granulosus* protoscoleces and completely eliminated the protoscoleces at the concentrations of 150 and 300 μL/mL (Alyousif et al., 2021).

Based on the peaks obtained in our GC/MS analysis, the main compounds were terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%). It has been previously proven that the main compounds of the essential oil of *Ferula* spp. are terpenoid composites, e.g., terpinolene, α-terpineol, α-pinene, β-pinene, myrcene, and other similar compounds (Sahebkar and Iranshahi, 2011). Based on the findings of previous studies, in essential oils, the chemical compositions are directly related to aspects such as the place and time of the collection of the plant, as well as the method and manner of extracting essential oils (Delfani et al., 2017). Terpenes and terpenoids are hydrocarbon compounds with various pharmacological and therapeutic properties, which especially act as antimicrobial agents against a wide spectrum of bacteria, fungal, viral, and parasitic strains (Guimarães et al., 2019; Mahizan et al., 2019). Considering the terpinolene-rich essentials, Ramos et al. (2014) have demonstrated that essential oils of *Mangifera indica* var. Espada and *M. indica* var.

Table 3
Determining the nitric oxide (NO) production in J774-A1 macrophage cells after treatment with various concentration of *Ferula macrecolea* essential oil and its main compound, terpinolene compared with the lipopolysaccharide (LPS, 10 ng/mL) along with IFN-γ (10 U/mL) as the positive control. Mean ± standard deviation (n = 3). IC_{50}: the 50% inhibitory concentrations.

Drug	Concentration (μg/mL)	NO production (μM)
Essential oil	¼ IC50	9.6 ± 2.58
	½ IC50	16.3 ± 2.63*
	IC50	22.6 ± 2.56 *
Terpinolene	¼ IC50	13.2 ± 2.01
	½ IC50	17.54 ± 1.86*
	IC50	26.12 ± 3.12 *
Non-treated	–	3.14 ± 0.31
IFN-γ + LPS	–	40.21 ± 2.65

* $P < 0.001$ significant difference compared with non-treated macrophage cells.

Rosa had potent leishmanicidal effects against promastigotes forms of L. *amazonensis* with IC_{50} of 39.1 and 23.0 μg/mL, respectively. Mikus et al., (2000) have reported the anti-parasitic effects of a number of isolated mono-and susquiterpenes against the growth rate of blood stream forms of *Trypanosoma brucei* and promastigotes of L. *major*. They reported that terpinen-4-ol had promising anti-parasitic effects against blood stream forms of *T. brucei* and promastigotes of *L. major* with IC_{50} values of 0.02 and 335.9 μg/mL, respectively; where these values were 31.0 and 387.9 μg/mL for terpinolene (Ramos et al., 2014; Mikus et al., 2000). Based on available studies, as their mechanisms of action, terpene and terpenoid compounds exhibit their antimicrobial effects by acting on the disruption of the cell wall, disruption of oxygen consumption, and inhibition of virulence factors, among others (Guimarães et al., 2019; Mahizan et al., 2019; Srivastava and Singh, 2019). According to previous studies, it is inferred that the anti-leishmaniasis effects of the *F. macrecolea* essential oil can also be due to the large amount of these compounds in it.

Evidence suggests that the prevention of infection in immune cells, especially macrophages, is one of the most important mechanisms targeted by drugs to control the *Leishmania* parasite (Albalawi et al., 2021b). Our results showed that the pre-incubation of *Leishmania* parasites with *F. macrecolea* essential oil and its main compound, terpinolene, significantly declined ($p < 0.001$) the rate of cell infection by 74.8% and 79.4%, respectively, when compared with non-treated parasites. One of the most important mechanisms of action of drugs on pathogenic microbes is their effect on cell wall permeability or its disruption (Albalawi et al., 2021b). The results revealed that by increasing the concentration of the essential oil and especially its main compound, terpinolene, the permeability of parasites changed significantly compared to the control group in a dose-dependent manner. This finding indicated that the essential oil and terpinolene can cause dysfunction and eventually cell death by having a direct effect on cell membrane permeability in the *Leishmania* parasite.

It has been demonstrated that NO-dependent cytotoxic effects induced by activated macrophages can eliminate and control intracellular pathogens such as *Leishmania* parasites (Mahmoudvand et al., 2016; Ashrafi et al., 2019; Adams, 2004b; Moazeni et al., 2014; Moazeni et al., 2019). In addition, in vivo studies have revealed that NO-inducing agents and agents that increase NO production are very promising for the treatment of cutaneous and visceral leishmaniasis in animals (Moazeni et al., 2019; Niazi et al., 2019). Our findings demonstrated that although more NO in macrophage cells was produced by increasing the concentrations of the essential oil, the amount of NO production in macrophage cells treated with the essential oil, especially its main compound, terpinolene, was significantly elevated ($p < 0.001$) when compared with the control group. In terms of cytotoxicity, our results revealed that the CC_{50} values for the essential oil, terpinolene, and glucantime were 471.3, 207.3, and 1165.3 μg/mL, respectively. Accordingly, the calculated SI of >10 for *F. macrecolea* essential oil, terpinolene, and glucantime indicated their specificity to amastigotes with minimum toxicity on macrophage cells.

5. Conclusion

The results of the present study showed that *F. macrecolea* essential oil, especially its main compound, terpinolene, has a potent antiparasitic effect on the promastigote and amastigote stages of *L. tropica*. Considering the advantages of medicinal plant products over their chemical counterparts, it is suggested that in the continuation of this study, the effect of *F. macrecolea* essential oil, especially terpinolene, on laboratory animals and in case of high efficiency, in humans be evaluated.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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None.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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