Identification of trans-2-cis-8-Matricaria-ester from the Essential Oil of *Erigeron multiradiatus* and Evaluation of Its Antileishmanial Potential by in Vitro and in Silico Approaches

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1. INTRODUCTION

Visceral leishmaniasis (VL) is caused by a protozoan parasite *Leishmania donovani*, which causes a fatal systemic infection. The disease is transmitted by a female sand fly (*Phlebotomus*). It is endemic in 62 countries of the developing world, and with an estimated population of 200 million at risk. Every year more than 100,000 new cases of VL are reported in India, and with an estimated population of 200 million at risk. Every year more than 100,000 new cases of VL are reported in India, and with an estimated population of 200 million at risk.

The control of leishmaniasis is not available yet due to the lack of effective vaccines and the disease control relies on chemotherapy with pentavalent antimonials which require long-term treatment and cause serious side effects. Some of the latest drugs such as miltefosine and liposomal amphotericin B are effective but are extortionate. Along with the toxicity and severe side effects, numerous relapse are also a major concern. Thus based on the present clinical scenario it is desirable to develop new antileishmanial agents from natural products which are productive, cost effective and less toxic.

The use of traditional medical practices, their validation and discovery of natural drugs could provide a new dimension for the treatment and control of leishmaniasis. Different aerial and underground parts of plants are commonly used to isolate essential oils (EOs) in folk medicine to treat various types of diseases. Recently antileishmanial properties have also been explored using EOs of *Cymbopogon citratus* (DC) Stapf (Poaceae), *Chenopodium ambrosioides* L. (Chenopodiaceae), and *Vanillosmopsis arborea* (Gardner) Baker (Asteraceae). *Erigeron multiradiatus* (Lindl.ex DC.) Benth. & Hook.f. is a natural inhabitant of the mountainous regions of India, Nepal, China, and Afghanistan. *E. multiradiatus* also known by its vernacular name “meiduoluomi” by traditional healers and native people, have been extensively
used to cure hepatitis, meningitis, hemiparasites, enuresis, diarrhea, adenolymphtis, rheumatism, and polyneuritis in traditional Tibetan medicine.\(^\text{11,12}\) Further, there are several reports on *Erigeron* spp. viz. *Erigeron canadensis* having antibacterial properties,\(^\text{13}\) *Erigeron annuus* with antimicrobial activity,\(^\text{14}\) *Erigeron floribundus* with antitypanosomal\(^\text{15}\) and antimalarial activity,\(^\text{16}\) and *Erigeron brevicaulis* widely used in Cameroonian traditional medicine, reported with analgesic, immunomodulatory, trypanocidal, antidermatophytes, anti-inflammatory, antifungal, and antimalarial activity.\(^\text{15,17–21}\) Moreover, the role of *Erigeron* spp. have also been reported to inhibit platelet aggregation, improve microcirculation, dilation of blood vessel, increase cerebral blood flow,\(^\text{22–24}\) to treat dental pain, angina, headache, female infertility, and AIDS.\(^\text{17,18,25–27}\)

More importantly, *E. multiradiatus* having antiadipic and anti-inflammatory activity,\(^\text{11,28}\) but no study has been reported on its antileishmanial potential to support their traditional folk medicinal use. *E. multiradiatus* collected from various regions unlocked the existence of several bioactive constituents namely matricaria ester, \(\alpha\)-pinene, lachnophyllum ester, myrcene, \(\beta\)-(E)-ocimene, isoledene, \(\alpha\)-copaene, \(\beta\)-cubebene, \(p\)-mentha triene, caryophyllene oxide, \(\alpha\)-cadinol, camphene, limonene, and \(\beta\)-eudesmol.\(^\text{14,29,30}\)

In this study, we have reported the isolation of EO of *E. multiradiatus*, identification and purification of \(\text{trans-2-cis-8-matricaria-ester}\) and further evaluation of its cytotoxicity and antileishmanial potential against *L. donovani*. In addition, in silico screening of the components of EOs together with the miltefosine reference antileishmanial agent was done on four vital parasitic enzymes, namely, \(\text{i-asparaginase-1-like protein, metacaspase-2, metacaspase-1, and DNA topoisomerase II}\) in order to find out its probable mode of action.

2. RESULTS AND DISCUSSION

2.1. EO Composition. The EO was isolated in a yield of 0.12% (v/w) from different aerial parts of *E. multiradiatus* and was inspected using gas chromatography with flame ionization detector (GC-FID) and gas chromatography–mass spectrometry (GC–MS) (Figure 1A). A sum of 12 components, corresponding to 97.81% of the total oil has been determined. The retention index of volatile compounds (RI\(^a\) and RI\(^b\)) and their percentage are summarized in Table 1. Among these, \(\text{trans-2-cis-8 matricaria ester}\) was identified as a major compound (77.79%) (Figure 1B) which was further confirmed by using mass, \(^1\text{H NMR}\) and \(^{13}\text{C NMR}\) spectral data (Table S1 and Figures S1–S3). The EO revealed the dominant existence of oxygenated monoterpenes (88.95%) followed by sesquiterpene hydrocarbons (5.61%) and oxygenated sesquiterpenes (3.05%). The monoterpane hydrocarbons accounted only for 0.20%. The other major components are \(\text{cis-lachnophyllum ester}\) (11.04%), \(\text{zingiberene}\) (4.43%), and \(\text{spathulenol}\) (1.59%) (Figure 2). In an earlier study of *E. multiradiatus*, \(\text{trans-2-cis-8 matricaria ester}\) (50.70%) was found as a main constituent.\(^\text{14}\) The retention index of volatile compounds (RI\(^a\) and RI\(^b\)) and corresponding to 97.81% of the total oil has been determined.

The number of viable promastigotes was significantly reduced (\(P < 0.001\)) in all tested concentrations (5, 10, 15, 20, 25, 30 \(\mu\text{g/mL}\) of EO as well as \(\text{trans-2-cis-8-matricaria ester}\) (5, 10, 25, 50, 75, 100 \(\mu\text{M}\)) in comparison to the untreated control (the coefficient of the variation range was 3–11%). Miltefosine as a positive control almost completely inhibited parasite proliferation (Figure 3A).

2.2. In Vitro Antileishmanial Activity. None of the promising therapeutic treatments are available against fatal VL. Therefore, it is an urgent need to identify the promising antileishmanial candidate. In the search of new potent antileishmanial agents, we have used the in vitro and in silico approach in this study extensively.

2.2.1. EO and \(\text{trans-2-cis-8-Matricaria Ester Inhibit the Growth of L. donovani Promastigotes}\). The effect of EO and \(\text{trans-2-cis-8-matricaria ester}\) was evaluated against the promastigote form of *L. donovani* (Dd8) by MTT assay, which significantly reduced *L. donovani* viabilities with \(\text{IC}_{50}\) values < 20 \(\mu\text{g/mL}\) and <56 \(\mu\text{M}\), respectively. Incubation of promastigotes with 30 \(\mu\text{g/mL}\) of EO and 100 \(\mu\text{M}\) of \(\text{trans-2-cis-8-matricaria ester}\) resulted in 80.95 ± 3.00 and 91.32 ± 8.48% inhibition, respectively. While at a concentration of 20 \(\mu\text{g/mL}\) or 50 \(\mu\text{M}\) the inhibition was 57.7 ± 6.08 and 48.3 ± 4.98%, respectively in promastigotes (Table 2). The \(\text{IC}_{50}\) value of EO against promastigotes was 18.29 ± 2.1 \(\mu\text{g/mL}\) and that for \(\text{trans-2-cis-8-matricaria ester}\) was 55.09 ± 6.4 \(\mu\text{M}\) (Table 3).

The number of viable promastigotes was significantly reduced (\(P < 0.001\)) in all tested concentrations (5, 10, 15, 20, 25, 30 \(\mu\text{g/mL}\) of EO as well as \(\text{trans-2-cis-8-matricaria ester}\) (5, 10, 25, 50, 75, 100 \(\mu\text{M}\)) in comparison to the untreated control (the coefficient of the variation range was 3–11%). Miltefosine as a positive control almost completely inhibited parasite proliferation (Figure 3A).

2.2.2. Investigation of Possible Cytotoxic Effects of EO and \(\text{trans-2-cis-8-Matricaria Ester on Hamster Peritoneal Macrophage}\). Different concentrations of EO and the purified

![Image](https://example.com/image1.png)
compound trans-2-cis-8-matricaria ester exhibited no cytotoxicity on hamster peritoneal macrophages; even the higher concentrations were safe without the sign of cell deformity. The CC50 value of EO against peritoneal macrophages was 285 ± 21 μg/mL and that for trans-2-cis-8-matricaria ester was 609 ± 71 μM (Table 3). Further, the safety of the therapeutic agents was evaluated by the selectivity index (SI), which was expressed by the CC50/IC50 ratio. The SI value for EO and trans-2-cis-8-matricaria ester was higher than 10 (Table 3), so the treatment was considered as safe for the cells (hamster peritoneal macrophages) at different therapeutic concentrations.

2.2.3. EO and trans-2-cis-8-Matricaria Ester Inhibit the Growth of L. donovani Intracellular Amastigote Forms. To check the activity of EO and trans-2-cis-8-matricaria ester on intracellular amastigotes, hamster peritoneal macrophages were infected with L. donovani and allowed to grow at different concentrations of EO and trans-2-cis-8-matricaria ester. The

Table 1. Chemical Composition of EO from E. multiradiatus

| S. No. | compound                          | RI<sup>a</sup> | RI<sup>b</sup> | % composition | method of identification |
|-------|-----------------------------------|----------------|---------------|----------------|--------------------------|
| 1     | β-(E)-ocimene                     | 1045           | 1050          | 0.20           |                          |
| 2     | preisopherfol-7-ene               | 1336           | 1336          | 0.15           |                          |
| 3     | methyl ergenol                    | 1400           | 1403          | 0.12           |                          |
| 4     | α-trans-bergamotene               | 1430           | 1434          | 0.92           |                          |
| 5     | α-humulene                        | 1451           | 1454          | 0.11           |                          |
| 6     | zingiberene                       | 1489           | 1493          | 4.43           |                          |
| 7     | cis-lachnophyllum ester           | 1527           |               | 11.04          |                          |
| 8     | trans-2-cis-8-matricaria-ester    | 1545           |               | 77.79          |                          |
| 9     | spathulene                        | 1578           | 1578          | 1.59           |                          |
| 10    | humulene epoxide II               | 1606           | 1608          | 0.34           |                          |
| 11    | cubenol                           | 1623           | 1628          | 0.25           |                          |
| 12    | α-cadinol                         | 1636           | 1640          | 0.87           |                          |
|       | total identified                  |                |               | 97.81%         |                          |
|       | monoterpene hydrocarbons          |                |               | 0.20%          |                          |
|       | oxygenated monoterpenes           |                |               | 88.95%         |                          |
|       | sesquiterpene hydrocarbons        |                |               | 5.61%          |                          |
|       | oxygenated sesquiterpenes         |                |               | 3.05%          |                          |
|       | total unidentified                |                |               | 2.19%          |                          |
|       | oil yield (% v/w)                 |                |               | 0.12           |                          |

<sup>a</sup>RI calculated relative to the homologous series of n-alkanes (C₈−C₃₄) on a Rtx-5 non-polar fused silica capillary column. <sup>b</sup>RI Adams. <sup>c</sup>MS, NIST, and WILEY libraries spectra and the literature. <sup>d</sup>(1H NMR, 13C NMR, COSY, HMBD and DEPT135) NMR spectra.

Figure 2. Structure of major components of EO from E. multiradiatus used in the molecular docking study.

Table 2. Effect of EO and trans-2-cis-8-Matricaria Ester of E. multiradiatus on L. donovani Promastigotes and Intracellular Amastigotes

| test sample/drug          | activity against promastigote | activity against intracellular amastigote |
|---------------------------|-------------------------------|------------------------------------------|
|                           | doses     | % inhibition (mean ± SD) | doses     | % inhibition (mean ± SD) |
| EO (μg/mL)                | 05        | 15.4 ± 1.86              | 05        | 12.4 ± 1.47              |
|                           | 10        | 24.8 ± 2.08              | 10        | 21.8 ± 2.12              |
|                           | 15        | 40.5 ± 5.00              | 15        | 35.5 ± 3.44              |
|                           | 20        | 57.7 ± 6.1               | 20        | 52.7 ± 6.7               |
|                           | 25        | 69.3 ± 7.4               | 25        | 61.3 ± 7.2               |
|                           | 30        | 80 ± 9.8                 | 30        | 75 ± 8.3                 |
| trans-2-cis-8-matricaria ester (μM) | 05 | 6.2 ± 0.86 | 05 | 4.8 ± 0.47 |
|                           | 10        | 11.6 ± 2.08              | 10        | 9.1 ± 1.12               |
|                           | 25        | 32.2 ± 3.00              | 25        | 23.9 ± 2.44              |
|                           | 50        | 48.3 ± 5.72              | 50        | 41.5 ± 4.89              |
|                           | 75        | 61.3 ± 7.9               | 75        | 58.9 ± 6.1               |
|                           | 100       | 91.7 ± 10.67             | 100       | 85.1 ± 8.9               |
| miltefosine (μM)          | 3.6       | 92.79 ± 1.17             | 3.6       | 82.31 ± 3.19             |

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number of amastigotes/100 macrophages was counted microscopically, and the results were expressed as a percentage inhibition as compared to the control (Figure 3B,C). Interestingly all the tested concentrations of EO (5, 10, 15, 20, 25, 30 μg/mL) and trans-2-cis-8-matricaria ester (5, 10, 25, 50, 75, 100 μM) were found to inhibit amastigote growth and resulted in 12.40 ± 1.47 to 75.00 ± 8.30% and 4.8 ± 0.47 to 85.10 ± 8.90% inhibition, respectively (p < 0.001), while the positive control (3.6 μM) lowered the parasite load in the infected macrophages by 82.31% (p < 0.001) compared to the untreated controls (Table 2). The IC50 value of EO against intracellular amastigotes was 20.19 ± 2.4 μg/mL and for trans-2-cis-8-matricaria ester was 61.2 ± 7.9 μM (Table 3). Different concentrations of the EO and trans-2-cis-8-matricaria ester were nontoxic to the hamster peritoneal macrophages and effective on intracellular amastigotes which indicated the selectivity of EO and trans-2-cis-8-matricaria ester against amastigotes as compared to mammalian cells, as analyzed by qualitative microscopic examination. Thus, the outcome signifies the selectivity of EO and its major component trans-2-cis-8-matricaria ester toward amastigotes as compared to the host cells.

As it is evident from the results, EO and its component trans-2-cis-8-matricaria ester provided a hindrance against both the promastigote and intracellular amastigote forms of L. donovani. This plant has antileishmanial potential which is almost similar to the standard drugs and possesses several medicinal properties, and because of easy oral administration, it could provide a good lead over currently available medicinal properties, and because of easy oral administration, it could provide a good lead over currently available (Figure 4). SiteMap gives knowledge about the binding sites in the receptor protein. The site score for all 10 protein binding sites of EO and trans-2-cis-8-matricaria ester is 0.80. In order to predict the effect of test compounds on different leishmanial proteins, we performed molecular docking interaction analysis (Table 4). The docking score corresponds to its free energy involved in “binding”. The entire attribute and genuineness of the model were found appropriate for the study. Molecular docking study revealed that trans-2-cis-8-matricaria ester was the only ligand that performed phenomenal binding affinity against four different protein molecules (Figure 5). Figure 5A–D represents interaction of ligands that manifest various interacting amino acid residues at the ligand-binding site of a protein. In Figure 5A, hydrogen bonding was clearly observed between the ligand and the ASP 117 and ARG 195 residue of L. donovani (Figure 5B–D). The higher the hydrogen bond, the stronger will be the interaction between the ligand and receptor. Other than trans-2-cis-8-matricaria ester, no other ligand showed activity and interaction with any proteins. The binding energy score of trans-2-cis-8-matricaria ester was −4.803, −4.152, −4.083, and −3.741 kcal/mol for L. donovani 1-asparaginase-1-like protein, metacaspase 2, metacaspase 1, and DNA topoisomerase II, respectively, which was significantly higher than the positive control miltefosine with a docking score of −3.835, −3.821, −3.495, and −3.158 for 1-asparaginase-1-like protein, metacaspase 2, metacaspase 1, and DNA topoisomerase II, respectively. Thus the results manifest that trans-2-cis-8-matricaria ester showed the highest binding affinity for 1-asparaginase-1-like protein while the binding energy of metacaspase-2, metacaspase-1, and DNA topoisomerase II were stronger than other proteins. Metacaspase

### Table 3. Antileishmanial Activity (IC50 ± sd) and Cytotoxicity Activity (CC50 ± sd) of EO and trans-2-cis-8-Matricaria Ester and the Positive Control

| test sample/drug | IC50 ± sd (promastigotes) | IC50 ± sd (intracellular amastigotes) | CC50 ± sd (hamster peritoneal macrophage) | SI-promastigotes (MQ/pro) | SI-amastigotes (MQ/ama) |
|------------------|---------------------------|-------------------------------------|-----------------------------------------------|--------------------------|--------------------------|
| EO (μg/mL)       | 18.29 ± 2.1               | 20.19 ± 2.4                         | 285 ± 21                                      | 15.58                    | 14.11                    |
| trans-2-cis-8-matricaria ester (μM) | 55.09 ± 6.4              | 61.2 ± 7.9                          | 609 ± 71                                      | 11.05                    | 10                       |
| miltefosine (μM) | 3.7 ± 0.4                 | 4.4 ± 0.2                           | 44.5 ± 7.9                                    | 12.02                    | 10.1                     |

aData (mean ± standard deviation) represents results of three independent experiments.
macrophages was determined microscopically after incubation with control. The percentage of intracellular amastigotes per 100 control group after subtracting the blank absorbance from the sample as the proportion of absorbance values normalized to the untreated varying concentrations of test compounds. Cell viability was expressed promastigotes was determined by MTT assay after 72 h exposure with or the number of amastigotes per 100 macrophages. The % viability of macrophages. The bar diagrams show the % viability of promastigotes (A) In vitro efficacy of different concentrations of EO, EO w/o trans-2-cis-8-matricaria ester and purified trans-2-cis-8-matricaria ester of E. multiradiatus against L. donovani promastigotes (B) intracellular amastigotes and (C) the percentage of infected macrophages. The bar diagrams show the % viability of promastigotes or the number of amastigotes per 100 macrophages. The % viability of promastigotes was determined by MTT assay after 72 h exposure with varying concentrations of test compounds. Cell viability was expressed as the proportion of absorbance values normalized to the untreated control group after subtracting the blank absorbance from the sample and control. The percentage of intracellular amastigotes per 100 macrophages was determined microscopically after incubation with different concentrations of the test compounds or the positive control miltefosine (3.6 μM) for 72 h. There were three replicates in each experiment, and the data are the mean ± SD for each concentration. Significance values indicate the difference between the untreated groups and treated groups with various concentrations of EO, trans-2-cis-8-matricaria ester, or miltefosine (* * * *, p < 0.001; **, p < 0.01; *, p < 0.05).

is essential for chromosomal separation and survival of the parasite. DNA topoisoerases are the key enzymes that facilitate high precision DNA transactions inside the parasite, and l-asparaginases are associated with the survival of the parasite. The major EO component, trans-2-cis-8-matricaria ester represents a good inhibitory effect on the four major essential parasite proteins with nontoxic properties. So these enzymes could be used as a potential drug target against the pathogen Leishmania.

2.3.2. ADME Profile of the Test Ligand. Pharmacokinetic comparative analysis of the test ligand depicts the fitness of the ligand as a drug as compared to the standard drug miltefosine (Table 5). Most of the absorption parameters suggest that the solubility and permeability of the ligand be in accordance with the control's value. Distribution parameters include renal organic cation transporters, P-glycoprotein and plasma protein binding (log PPB). Metabolism data included the substrate and inhibition analysis of various drug metabolism enzymes belonging to the CYP450 enzyme superfamily. Excretion parameters include Madin-Darby canine kidney (MDCK) cell lines on in silico levels.

3. CONCLUSIONS

In summary, we have identified the chemical components of EO of E. multiradiatus and evaluated its antileishmanial potential against L. donovani. The results show that the EO of E. multiradiatus exhibits leishmanicidal activity in vitro against L. donovani and that this activity is related to the presence of its major compound trans-2-cis-8-matricaria ester. This major compound, trans-2-cis-8-matricaria ester, manifests more than 77% of the composition of EO, and when tested separately, it also exhibited similar or more leishmanicidal activity. Further, in silico study revealed that trans-2-cis-8-matricaria ester may be an effective inhibitor of the four pathogenic proteins of Leishmania with nontoxic properties that could be further employed for in vivo examination and may enhance the pace of herbal drug development, which may be a better option for alternate chemotherapy against VL.

4. EXPERIMENTAL SECTION

4.1. Plant Materials. The plant material was collected in the month of September 2017 (flowering stage) from the Chipilakedar forest (Pithoragarh District), Uttarakhand, India, at an altitude of 3000 m with geographical coordinates 29° 96′ N latitudes and 80° 43′ E longitudes. A voucher (specimen no. 116031) has been deposited at the Herbarium of Botanical Survey of India, Dehradun, India; and the Department of Chemistry, Kumaun University, Almora, India.

4.2. Extractions of the EO. Fresh aerial parts (~4 kg) were exposed to steam distillation using a copper still distiller (Scientech, India). The distillate of fresh plant material was treated with n-hexane and dichloromethane for thorough extraction of organic components. The dichloromethane and n-hexane extracts were mixed and dried over anhydrous Na2SO4. In order to obtain residual oil, solvent distillation was
performed in a rotary vacuum evaporator (Perfit-RV 1240, Buchi type, India). Further using anhydrous sodium sulphate the oil was allowed to dry, filtered and stored at 4 °C until its chemical and pharmacological test analysis.

4.3. GC-FID and GC–MS Analysis. A gas chromatographic analysis of EO was performed on a Shimadzu GC-2010 Ultra gas chromatograph, Kyoto, Japan, equipped with a flame ionization detector and an Rtx-5MS fused silica capillary column. The temperature of the injector and detector were maintained at 260 and 270 °C, respectively. Helium at a flow rate of 1.21 mL/min and 69.0 kPa inlet pressure was employed as the carrier gas. The sample (1.0 μL) was injected with 10:1 split ratio.

GC–MS was carried out on Shimadzu GC-MS-QP2010 Ultra, Kyoto, Japan, using identical oven temperature. The MS was used under the electron impact conditions (70 eV), ion 230 °C, mass scan mode: 2.41 scan/s, mass range: 40−650 m/z; a 5% solution of oil in hexane (1.0 μL) was injected. Individual compounds were identified by calculating retention indices (RI) using homologous C8−C34 (Supplier: Restek’s ISO 9001:2008) n-alkane series, compared with available mass spectral data (NIST 11, Wiley 8 and FFNSC 2) and finally confirmed by comparing their RI with the available literature.37

4.4. Isolation and Characterization of trans-2-cis-8-Matricaria Ester. The EO (2 mL) of E. multiradiatus was subjected to a silica gel column chromatograph (230−400 mesh, Merck, 20 g) with hexane/diethyl ether (99:1−85:15) as the eluent and twenty fractions were collected and screened by TLC and GC to produce the compound (80 mg, >96% purity). The compound was identified by using mass,1H NMR and 13C NMR spectral data.37

4.5. Parasite and Media. Promastigotes of L. donovani (Dd8) were cultured in RPMI-1640 medium (Himedia, India) containing 10% fetal bovine serum (Cell clone, Genetix, India)
along with 1% antibiotic and antymycotic solution (Cell clone, Genetix, India) at 26 °C.

4.6. Animals. Laboratory inbred female golden hamsters (Mesocricetus auratus, 45–50 g) were purchased from the Central Drug Research Institute (CDRI-CSIR), Lucknow, India and were used for experimental purposes. All animals and experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the care and use of laboratory animals and ethics committee, and the protocol number is KUDOPS/109. They were kept in a climatically controlled animal house and fed with a standard rodent food pellet (Lipton India) and water ad libitum.

4.7. In Vitro Activity. 4.7.1. Evaluation of Inhibition of L. donovani Promastigotes Growth. To determine the efficacy of the drug log phase, L. donovani promastigotes were used. Briefly, \(10^5\) parasites/well were plated on 96-well cell culture plates along with different concentrations of EO and trans-2-cis-8-matricaria ester and incubated for 72 h at 26 °C. Then 20 \(\mu\)L of MTT (Himedia, India) stock solution (5 mg/mL) was mixed into each well and further incubated for 4 hours then allowed to centrifuge for 10 min at 1000 \(g\); the supernatant was discarded and resuspended with 100 \(\mu\)L (0.5%) of DMSO (Cell clone, Genetix, India) into each well. Using a microplate reader (Bio-Rad, India) the OD was measured at 540 nm. Further percentage of inhibition was calculated by comparing the % viability with the untreated control. In order to verify the results, tests were performed in triplicate. Miltefosine was used as a standard control.

4.7.2. Cytotoxicity of EO and trans-2-cis-8-Matricaria Ester in Hamster Peritoneal Macrophages. The cytotoxicity of EO and trans-2-cis-8-matricaria ester was evaluated on hamster peritoneal macrophages. Two hamsters were treated with thioglycollate in the peritoneal cavity to allow inflammatory response to proceed for 4 days and then euthanized. Hamster peritoneal macrophages were resuspended at \(10^6\) cells/mL in RPMI medium plated in 16-well

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Table 5. ADME Profile of the Test Ligand (trans-2-cis-8-Matricaria Ester) and Control (Miltefosine)

| parameter                      | test ligand | control |
|--------------------------------|-------------|---------|
| **Absorption**                 |             |         |
| log \(S\)                       | -2.15       | -2.29   |
| human oral absorption           | 92.65       | 100     |
| human intestinal absorption     | 93.51       | 97.28   |
| log BBB                         | 0.420       | 0.629   |
| rate of membrane permeability   | 31.91       | 85.73   |
| Caco-2 permeability             | 325.29      | 183.69  |
| **Distribution**                |             |         |
| renal organic cation transporter| non-inhibitor| non-inhibitor |
| \(P\)-glycoprotein substrate    | non-substrate| non-substrate |
| \(P\)-glycoprotein inhibitor    | non-inhibitor| non-inhibitor |
| log PPB                         | 2.0         | 0.9     |
| **Metabolism**                  |             |         |
| CYP450 2C9 substrate            | non-substrate| non-substrate |
| CYP450 2D6 substrate            | non-substrate| non-substrate |
| CYP450 3A4 substrate            | substrate    | non-substrate |
| CYP450 1A2 inhibitor            | non-inhibitor| non-inhibitor |
| CYP450 2C9 inhibitor            | non-inhibitor| non-inhibitor |
| CYP450 2D6 inhibitor            | non-inhibitor| non-inhibitor |
| CYP450 2C19 inhibitor           | inhibitor    | non-inhibitor |
| CYP450 3A4 inhibitor            | non-inhibitor| non-inhibitor |
| \(CYP\) inhibitory promiscuity  | low CYP inhibitory promiscuity | low CYP inhibitory promiscuity |
| **Excretion**                   |             |         |
| MDCK                            | 652.82      | 1282.43 |

Experiments on Animals (CPCSEA) for the care and use of laboratory animals and the regulations of Kumaun University, Nainital, India. The use of animals was approved by the institutional animal care and ethics committee, and the protocol number is KUDOPS/109. They were kept in a climatically controlled animal house and fed with a standard rodent food pellet (Lipton India) and water ad libitum.
chamber slides (Nalge Nunc, USA) and incubated for 8 days for differentiation into macrophages in a humidified 5% CO₂ air atmosphere at 37 °C. Further, the differentiated hamster peritoneal macrophages were incubated with various concentrations of EO and trans-2-cis-8-matricaria ester for 72 h. The cytotoxicity of compounds was examined using MTT assay, and cell morphology and integrity were evaluated under a microscope after Giemsa staining (Himedia, India). To calculate the cytotoxic concentration (CC₅₀), tests were conducted in triplicate. SI, which is the ratio of CC₅₀/IC₅₀, manifests the balance between cytotoxicity and antileishmanial activity. SI value > 10 is considered to be safe for the cells at various concentrations.

4.7.3. Evaluation of the Inhibitory Effect on Intracellular Amastigote Growth. Hamster peritoneal macrophages were seeded and infected with stationary-phase Leishmania promastigotes (at a ratio of 1:10 macrophages to parasites). The infected macrophages were subjected to grow for 72 h along with various concentrations of EO and trans-2-cis-8-matricaria ester. Sensitivity was checked microscopically after Giemsa staining by calculating the burden of amastigotes per 100 macrophages. Miltefosine was used as the standard positive control. Percentage of inhibition was calculated as described earlier.

4.8. In Silico Study. 4.8.1. Target Selection. The amino acid sequences of the target proteins of L. donovani cytoplasmonic l-asparaginase-1-like protein (CBZ32861.1), L. donovani metacaspase-2 (ABD19718.1), L. donovani metacaspase-1 (ABD19717.1), and L. donovani DNA topoisomerase II (AADD4021.1) were retrieved using the database of NCBI (www.ncbi.nlm.nih.gov/). For interaction analysis of the selected target proteins and ligands, Schrödinger’s software is used (Schrödinger Release: Maestro, version 10.5, Schrödinger, LLC, NY 2016-1, USA). This master software consists of more than one suite that is capable of performing visualization of the structure, binding site prediction, and receptor—ligand interaction.

4.8.2. Ligand Selection and Preparation. The ligands used in this study were selected from chemical components of E. multiradiatus EO (Figure 2). The receptors used are significant proteins, playing a major role in the infection cycle of L. donovani, hence can be used as attractive targets for targeting leishmaniasis. For ligand preparation, Ligprep application (v3.7, Schrödinger Release NY 2016-1, USA) was used for rectifying and stabilizing the structure of the ligand.

4.8.3. Protein Structure Generation. Structure of proteins involved in the study was either obtained from RCSB (protein databank) or generated using models. In the absence of 3D structures online, we employed the homology modeling approach using PRIME for modeling of 10 proteins. Hence, the amino acid sequences (FASTA) were retrieved from UniProt and are reported here (sequence 1). The template for sequences was generated using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the template selected were further modeled to a 3D structure by Prime (version 7.0, Schrödinger Release NY 2016-1, USA).

4.8.4. Protein Structure Validation. Determining the accuracy of the homology modeled 3D structure requires mandatory validation. This step is called preparation of protein that primarily includes minimization of energy, removal of water, and addition of hydrogen bonds. It is facilitated by Protein Preparation Wizard (version 7.0, Schrödinger Release NY 2016-1, USA). Validation of proteins 3D resulted in a refined model structure preceded by generating the binding sites on the target for the ligand to attach. The binding site for our target proteins were predicted using SiteMap (version 3.8, Schrödinger release NY 2016-1 USA).

4.8.5. Molecular Docking. A docking study was carried out to fit ligands and proteins into the predicted binding site of all the protein targets selected for the study. To evaluate the interaction and binding affinity between the ligand and receptor the Glide package was used (version 7.0, Schrödinger Release NY 2016-1, USA). The size of the docking area was prede fined by receptor grid generation as per the presence of the binding site in respective protein structures.

4.8.6. ADME Studies. The pharmacokinetic profile of the test ligand was analyzed in terms of its absorption, distribution, metabolism, and excretion. This ADME analysis was carried out by Qikprop (an application from Schrödinger suite 2016).

4.9. Statistical Analysis. All experiments were performed in triplicates and the results were manifested as mean ± SD. Analysis of the results (pooled data of the three experiments) were performed by one-way ANOVA followed by Dunnnett’s post-test. All of the analyses were done using GraphPad Prism (version 3.03) software.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02130.

NMR data of the compound trans-2-cis-8 matricaria ester in CDCl₃ (1H: 500 MHz; 13C: 125 MHz). (C) mass spectrum of trans-2-cis-8-matricaria ester, (D) ¹H NMR spectrum of trans-2-cis-8-matricaria ester. (E) ¹³C NMR spectrum of trans-2-cis-8-matricaria ester, (F) COSY spectrum of trans-2-cis-8-matricaria ester, (G) DEPT-135 spectrum of trans-2-cis-8-matricaria ester, (H) HMBC spectrum of trans-2-cis-8-matricaria ester (PDF)

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Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS**

EO, essential oil; FBS, fetal bovine serum; GC-FID, gas chromatography with flame ionization detector; GC-MS, gas chromatography–mass spectrometry; GC, gas chromatography; NMR, nuclear magnetic resonance; RPSI, Roswell Park Memorial Institute medium; TLC, thin layer chromatography; VL, visceral leishmaniasis

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