International Journal of Medical Laboratory 2019;6(2):131-137.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Antileishmanial Activity of *Carum Copticum* Essential Oil Against *Leishmania Major* [MRHO/IR/75/ER]: An *In Vitro* Study

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ABSTRACT

Article history
Received 8 Jul 2018
Accepted 6 May 2019
Available online 31 May 2019

Key words
Carum
Leishmania major
Leishmaniasis
Promastigotes

Background and Aims: Because of the toxicity and side-effects of synthetic drugs, there is a growing interest in biomedical plants. The aim of this study was to evaluate *in vitro* antileishmanial activity of *Carum copticum* essential oil against *Leishmania (L) major*.

Materials and Methods: Nineteen experimental groups were designed to determine the effect of *Carum copticum* essential oil against *L. major* and compare it with Meglumine antimonite. Group 1 was the control group and included 200 µl of RPMI 1640 plus 2×10^5 cells/ml promastigotes. Groups 2-10 included the aforementioned substances plus 10 µl of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 and 3 µg/ml of *Carum copticum* essential oil respectively. Groups 11-19 were similar to groups 2-10 but Meglumine antimonite (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 and 3 µg/ml) was used instead of *Carum copticum* essential oil. All the experiments were repeated five times. After 8 hours, the antileishmanial activities of studied substances were determined.

Results: Up to concentration of 0.5 µg/ml, no effect was observed with both substances. In comparison to control group, at 1 and 2 µg/ml, Meglumine antimonite had no effect on Leishmaniasis ($p>0.05$) while *Carum copticum* essential oil significantly decreased Leishmaniasis viability ($p<0.05$). Moreover, at 3 µg/ml, both compounds significantly decreased Leishmaniasis viability ($p<0.05$). However, *Carum copticum* essential oil had substantially better Antileishmanial activity than the other.

Conclusions: These results suggest that comparable concentrations, *in vitro* antileishmanial activity of *Carum copticum* essential oil is better than Meglumine antimonite.

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Introduction

Leishmaniasis is caused by protozoan parasites and belongs to the genus *Leishmania*. The parasites are transmitted by the bite of an insect vector, the phlebotomie sand-fly [1]. The most common form of it is cutaneous *Leishmania*sis which is an endemic disease in the southern parts of Iran [2]. It has been reported that 90% of cutaneous Leishmaniasis cases occurs in Afghanistan, Iran, Saudi Arabia and Syria [3]. The Leishmaniasis is usually a self-limiting disease, but may result in severe disfigurement. The manifestations could be greatly variable depending on the strain of parasite, the host's immunological status, or a secondary infection [4]. Current treatments include pentavalent Antimonials sodium Stibogluconate (Pentostam) and Meglumine antimonite (MA). These drugs require long courses of parenteral administration and have toxic side effects such as cardio toxicity, reversible renal failure, pancreatitis, anemia, leukopenia, rash, headache, abdominal pain, nausea, vomiting, arthralgia, myalgia, thrombocytopenia, and elevation of transaminase [5].

MA is the main therapy in the endemic regions because of its efficacy and cost effectiveness. The disadvantages of the Antimonials are their daily requirement for intramuscular or intravenous injection for 20-28 days, toxicity, and growing incidence of resistance in the endemic and non-endemic regions [6]. Other medications, like Pentamidine and amphotericin B, have been employed as alternative drugs [7]. Due to severe toxicity of these drugs, there is an increased tendency toward the use of natural sources because of their comparatively lower side effects and easier availability [8, 9]. Natural products could be the potential sources of compounds with anti-Leishmania activity and studying their functions may lead to discovering new drugs against this parasite [10].

The plant *Carum copticum* (*Trachyspermum ammi*) belongs to the Apiaceae family and is routinely used against flatulence, atonic dyspepsia, diarrhea, and often recommended for cholera [11]. Principal constituents of *Carum copticum* are phenols, mainly thymole and some carvacrol. Thymole is a powerful antiseptic and antifungal agent and is used in deodorant, mouthwashes and toothpastes [12]. In Persian traditional medicine, it has been used for centuries and different therapeutic implications for *Carum copticum* have been described [13]. Nagulakshmiet and his colleagues have reported antimicrobial and antibacterial activity for this plant [14]. Moreover, Ahmad Khan et al. showed more than 80% reduction in elastase activity of *A. fumigatus* MTCC2550 by *Carum copticum* and thymol [15]. Another study revealed that alcoholic extracts of it can have antileishmanial activity against *Leishmania major (L. major)* promastigotes [2].

Furthermore, it has been reported that *Carum copticum* has curative effect in the ulcer size and burden of cutaneous Leishmaniasis in the spleen of mice compared with Meglumine antimoniate [16]. Medicinal plant extracts or

plant derived compounds are potential alternatives to the present costly medicines against parasites [6]. Little information exists on Antileishmanial activity of *Carum copticum* essential oil (CCEO). The aim of the present study was to investigate the *in vitro* Antileishmanial activity of CCEO against *L. major* [MRHO/IR/75/ER].

Materials and Methods

Extraction procedure

Carum copticum was obtained from Herbal Medicinal Center at Shahid Sadoughi University of Medical Sciences. First, 550 g of air dried seeds of *Carum copticum* were gently grounded and mixed with 500 ml of double distilled water. Then it was extracted by the steam distilled apparatus. Afterwards, CCEO was separated from aqueous extract by soxhlet apparatus.

Parasite culture

L. major strain [MRHO/IR/75/ER] promastigotes were obtained from the Medical Parasitology department of Shahid Sadoughi University of Medical Sciences and maintained in BALB/c mice. Amastigote were separated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN). The third passage promastigotes from NNN medium were progressively adapted to Roswell Park Memorial Institute (RPMI) 1640 media (Sigma, St. Louis, MO, USA) and supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25˚C. Promastigotes forms in stationary phase were suspended in fresh RPMI 1640 medium to a final concentration of 4×10^6 cells/ml. The test was performed in 96-well microliter plates and each well was filled with 100 µl of culture medium. Then, plates were incubated at 27˚C for 1 h before adding CCEO [17].

Cell proliferation Assay Kit

After 8 hours, the anti-Leishmania bioassay was performed by XTT (Sigma, St. Louis, MO, USA) detecting kit using chemiluminescent assay. The nucleases are nitrogen-containing biological compounds including adenine, cytosine, guanine and thymine. Briefly, in this technique, the detector substance acts on the thymine base. XTT solution was prepared as 5 mg/ml in RPMI 1640 without phenol red and filtered through a 0.2 µm filter and 20 µl of this concentration was added to each well and incubated at 25˚C for 24 h. Then to solve the formazan crystals, 150 µl of acidic isopropanol was added to each well. The plate was read by an enzyme-linked immunosorbent assay (ELISA) reader using 450 nm as test wavelength and 630 nm as the reference wavelength [17].

Study groups

Nineteen groups were designated to study the effect of different concentrations of CCEO and compare it with MA. The study groups are listed in table 1. The experiment in each group was repeated 5 times.

Table 1. The study groups

Groups	
I	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes (Control)
II	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes + 10µl of 0.01 µg/ml CCEO
III	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 0.02 µg/ml CCEO
IV	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 0.05 µg/ml CCEO
V	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes + 10µl of 0.1 µg/ml CCEO
VI	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 0.2µg/ml CCEO
VII	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 0.5µg/ml CCEO
VIII	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of µg/ml CCEO
IX	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 2µg/ml CCEO
X	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 3µg/ml CCEO
XI	200µl of $RPMI_{1640}$+2×10^5cells/ml promastigotes+10µl of 0.01 µg/ml MA
XII	200µl of $RPMI_{1640}$ +2×10^5cells/ml promastigotes+10µl of 0.02µg/ml MA
XIII	200µl of $RPMI_{1640}$ +2×10^5cells/ml promastigotes+10µl of 0.05µg/ml MA
XIV	200µl of $RPMI_{1640}$ +2×10^5 cells/ml promastigotes +10µl of 0.1µg/ml MA
XV	200µl of $RPMI_{1640}$ +2×10^5 cells/ml promastigotes +10µl of 0.2µg/ml MA
XVI	200µl of $RPMI_{1640}$ +2×10^5 cells/ml promastigotes +10µl of 0.5µg/ml MA
XVII	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 1µg/ml MA
XVIII	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 2µg/ml MA
XIX	200µl of $RPMI_{1640}$ + 2×10^5 cells/ml promastigotes +10µl of 3µg/ml MA

Statistical analysis

All data are reported as mean±standard deviation. Statistical analysis was done using SPSS 21.0 for windows package. One-way analysis of variance (ANOVA) was performed followed by post-hoc LSD. The $p<0.05$ was considered to assess significant protection in treatment groups.

Results

The results of antileishmanial activity of CCEO on cutaneous Leishmaniasis are illustrated in figure 1. As it is shown, different levels of the CCEO up to 1 µg/mL had no effect on the viability of Leishmaniasis promastigotes as compared to control group ($p>0.05$). However, levels of 2 and 3 µg/ml of it could significantly decrease the viability of Leishmaniasis promastigotes ($p<0.05$).

Effect of different levels of MA compared to negative control group against viability of cutaneous Leishmaniasis promastigotes in the stationary phase is illustrated in figure 2. Different levels of the MA up to 2 µg/mL had no effect on the viability Leishmaniasis promastigotes in the stationary phase compared to control group ($p>0.05$). Nevertheless, concentration of 3 µg/ml of MA significantly decrease Leishmaniasis promastigotes ($p<0.05$). Comparison of CCEO and MA against viability of cutaneous Leishmaniasis in the stationary phase revealed that levels of 2 and 3 µg/ml of the CCEO significantly decreased Leishmaniasis viability in comparison to the similar concentrations of MA ($p<0.05$).

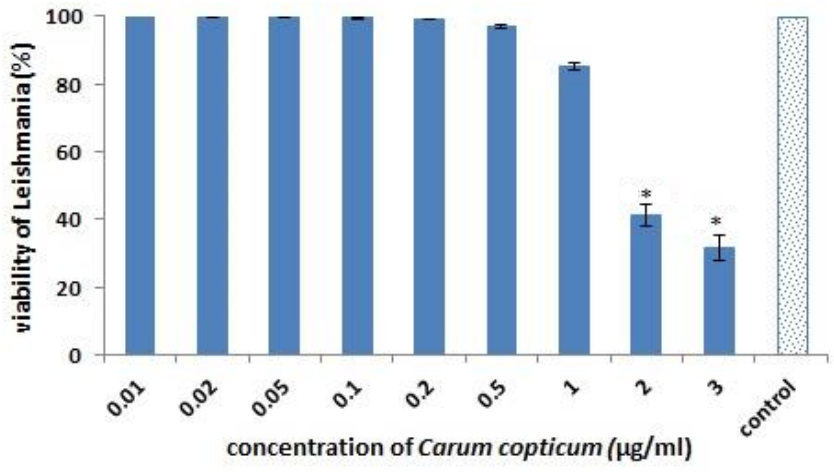

Fig. 1. Effect of different levels of *Carum copticum* essential oil compared to negative control against viability of cutaneous leishmaniasis promastigotes in stationary phase. *: p<0.001

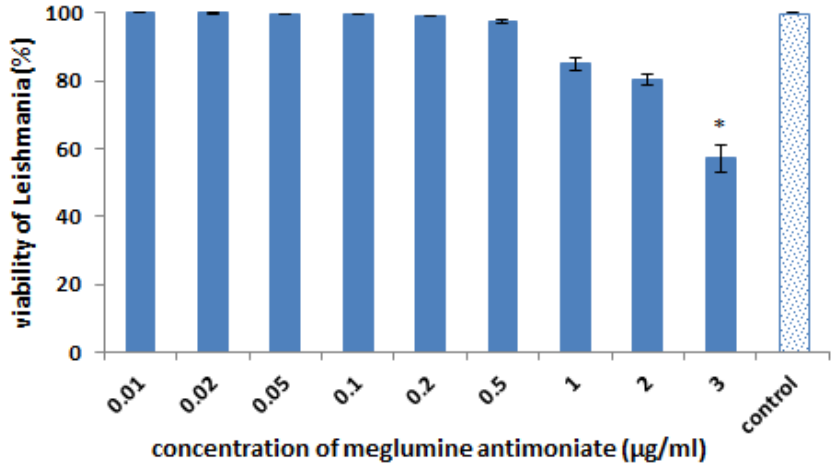

Fig. 2. Effect of different levels of Meglumine antimonites compared to negative control against viability Cutaneous Leishmaniasis promastigotes in stationary phase.*: p<0.05

Discussion

To our knowledge, limited information exists on the activity of CCEO against viability of cutaneous Leishmaniasis in comparison with MA. As it is observed, above 1 µg/ml, CCEO has better antileishmanial activity compared to MA in similar concentrations. Pentavalent antimonials like sodium Stibogluconate is the base for Leishmaniasis therapy in the endemic regions. Due to the limited availability of effective pharmaceutical drug and their side effects, usually patients in these areas largely tend to use popular treatments and traditional medicines to alleviate the symptoms [18].

Phytochemical analysis of *Carum copticum* has revealed the presence of terpenes and sterols. Also, GC/GC-MS analysis has shown that Thymole is the major component (97.5) of *Carum copticum*. Antioxidant activities of Thymole and carvacrol previously published have used various testing systems [19]. The anti-inflammatory effect of *Carum copticum* seed extract was also observed in a double

blind clinical study [20]. Furthermore, it has been reported that major components of essential oil of *Carum copticum* were Thymole (45.95%), γ-terpinene (20.6%), and o-cymene (19%) and to a lesser degree, ethylene methacrylate (6.9%), β-pinene (1.9%), and hexadecane (1.1%) are the other constituents of the plant [21]. Anthelmintic effect of *Carum copticum* in comparison with levamisole, an anthelminthic and immunomodulatory drug, on sheep infected with mixed nematode has also been evaluated. *Carum copticum* powder dose- dependently causes reduction in eggs per gram of feces which is more potent compared to levamisole [22]. *Plasmodium falciparum* is genus of parasitic protozoa. Infection with this genus is known as malaria. Ethyl acetate extract of *Carum copticum* seed at concentration of 25 μg/mL has also shown *in* vitro antimalarial activity [3].

Today, consumers are looking for high-quality products of natural origin [23]. Our study showed high efficacy of herbals against Leishmaniasis *in vivo.* For many years, *Carum copticum* has been used as an antibacterial, antifungal, and antiprotozoal agent. The alcoholic extract and the essential oil of *Carum copticum* were tested against *Giardia lamblia* cysts [24]. Beneficial effect of *Carum copticum* seeds in the treatment of Leishmaniasis parasitic has also been reported. Hydro-alcoholic extract of *Carum copticum* has shown antileishmanial activity with IC_{50} of 15.625 μM which was less than IC_{50} for macrophage cell line (43.76 μM) [13].

de Moaris and his colleagues demonstrated the anti-Leishmania activity of Thymole both *in vitro* and *in vivo* [25]. Therefore; it is likely that the anti-Leishmania activity of *Carum copticum* is mediated by cymene and Thymole components [6].

Conclusion

Based on our results, *Carum copticum* essential oil has better antileishmanial activity than MA. This study confirms that natural products are potential sources of new and selective agents that can contribute significantly to primary health care and probably are promising substitutes for current drugs used in the treatment of Leishmaniasis. However, further *in vivo* and *in vitro* research is needed to determine cellular and molecular effects of the *Carum copticum* in clinical trials.

Conflicts of Interest

There was no conflict of interest.

Acknowledgement

The authors would like to thank the authorities of the faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences for financial support.

References

[1]. Oliveira VCS, Moura DMS, Lopes JAD, De Andrade PP, Da Silva NH, Figueiredo RCBQ. Effects of EOs from Cymbopogon citratus (DC) Stapf., Lippia sidoides Cham., and Ocimum gratissimum L. on growth and ultrastructure of Leishmania chagasi promastigotes. Parasitol Res. 2009; 104(5): 1053-1059.

[2]. Tabatabaie F, keighobadi A, Golestani M, Shahmohammad N, Voosoogh M, Noori M, et al. Antileishmanial activity of Hyssopus

officinalis, Tussilago farfara, Carum copticum extracts in comparison with Glucantime in Iran. JMPS 2014; 2(5): 12-18.
[3]. Kheiri Manjili H, Jafari H, Ramazani A, Davoudi N. Antileishmanial and toxicity activities of some selected Iranian medicinal plants. Parasitol Res. 2012; 111(5): 2115-121.
[4]. Mishra BB, Kale RR, Singh RK, Tiwari VK. Alkaloids: future prospective to combat Leishmaniasis. Fitoterapia 2009; 80(2): 81-90.
[5]. Corrêa DS, Tempone AG, Reimão JQ, Taniwaki NN, Romoff P, Fávero OA, et al. Anti-leishmanial and anti-trypanosomal potential of polygodial isolated from stem barks of Drimys brasiliensis Miers (Winteraceae). Parasitol Res. 2011; 109(1): 231-6.
[6]. Kheiri Manjili H, Jafari H, Ramazani A, Davoudi N. Anti-leishmanial and toxicity activities of some selected Iranian medicinal plants. Parasitol Res. 2012; 111(5): 2115-121.
[7]. Pontin K, Da Silva Filho AA, Santos FF, Silva MLA, Cunha WR, Nanayakkara NPD, et al. In vitro and in vivo antileishmanial activities of a Brazilian green propolis extract. Parasitol Res. 2008; 103(1): 487-492.
[8]. Fattahi Bafghi A, Noorbala M, Noorbala MT, Aghabagheri M. Anti Leishmanial Effect of Zinc Sulphate on the Viability of Leishmania tropica and L. major Promastigotes. Jundishapur J Microbiol. 2014; 7(9): e11192.
[9]. Fattahi Bafghi A, Vahidi AR, Anvari MH, Barzegar K, Ghafourzadeh M. The in vivo antileishmanial activity of alcoholic extract from Nigella sativa seeds. Afr J Microb Res. 2011; 5(2): 1504-510.
[10]. Kayser O, Kiderlen A, Croft SL. Natural products as antiparasitic drugs. Parasitol Res. 2003; 90(1): S55-62.
[11]. Zarshenas MM, Samani SM, Petramfar P, Moein M. Analysis of essential oil components from different Carum copticum L. components samples from Iran. Pharmacognosy Res. 2014; 6(1):62-6.
[12]. Krishnamoorth V, Mada LMB. Trachy-spermum ammian essential crop for North Karnataka. J medic aromatic plant sci. 1999; 4: 996-98.
[13]. Boskabady MH, Alitaneh S, Alavinezhad A. Carum copticum L.: A herbal medicine with various pharmacological effects. BioMed Res Int. 2014; 2014: 569087.
[14]. Nagulakshmi S, Shankaracharya NB, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan aspects (Trachyspermum ammi). J Food Sci Techol Mysore. 2000; 39: 277-81.
[15]. Ahmad Khan SM, Ahmad I, Cameotra SS. Carum copticum and Thymus vulgaris oils inhibit virulence in Trichophyton rubrum and Aspergillus spp. Braz J Microbiol. 2014; 45(2): 523-531.
[16]. Akhlaghi L, Yeganeh M, Maleki F, Golestani M, Noori M, Ghelman M, et al. Antileishmanial Activity of Hyssopus officinalis, Tusasilage farfara, Carum copticum extracts in Mice Infected with Leishmania major . Int J Herb Med. 2014; 2(2): 142-145.
[17]. Fouladvand M, Barazesh A, Farokhzad F, Malekizadeh H; Sartavi K. Evaluation of in vitro anti-Leishmanial activity of some brown, green and red algae from the Persian Gulf. Eur Review Med Pharmacol Sci. 2011; 15(6): 597-600.
[18]. Kermanjani A, Jalalizadegan B, Tabatabaie F. Comparison of Hyssopus officinalis, Tussilage farfara, Carum copticum extracts versus systemic Glucantime in the treatment of cutaneous leishmaniasis in Balb/c mice. Adv Stud Biol. 2015;7(2): 49-54
[19]. Altiok D, Altiok E, Tihminlioglu F. Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. J Mater Sci Mater Med. 2010; 21(7): 2227-236.
[20]. Thangam C, Dhananjayan R. Antiinflam-matory potential of the seeds of Carum copticum Linn. Indian J Pharmacol. 2003; 35: 388-391.
[21]. Mahboubi M. Kazempour N. Chemical composition and antimicrobial activity of Satureja hortensis and Trachyspermum copticum essential oil. Iran J Microbiol. 2011; 3(4): 194-200.
[22]. Lateef M, Iqbal Z, Rauf U, Jabbar A. Anthelmintic activity of Carum copticum seeds against gastro-intestinal nematodes of sheep. J Anim Pl Sci. 2006; 16(1-2): 34-37.
[23]. Kadam, SU, Tiwari BK, O'Donnell CP. Application of novel extraction technologies for bioactives from marine algae. J Agric Food Chem. 2013; 61(20):4667-675.
[24]. Shahabi S, Ayazi RF, Kamalinejad M, Abadi. Anti-giardia activity of Carum copticum on Giardia lamblia cysts in vitro. Pejouhesh Dar Pezeshki 2008; 32(4): 303-307.
[25]. de Morais SM, Vila-Nova NS, Bevilaqua CM, Rondon FC, Lobo CH, Moura AD, et al. Bioorganic and medicinal chemistry thymol and eugenol derivatives as potential antileishmanial agents. Bioorg Med Chem. 2014; 22(21): 6250-255.