Chemical characterization and anti-parasitic property of essential oil of Coriandrum sativum leaf against Trichomonas vaginalis

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ABSTRACT
Coriandrum sativum has been used in Iranian traditional medicine as an anti-inflammatory, antioxidant, antibacterial, and antifungal agent. The purpose of this study was to evaluate the chemical composition and anti-parasitic property of essential oil of C. sativum leaf on trophozoite of Trichomonas vaginalis. C. sativum was collected from Kermanshah city and essential oil was prepared by the Clevenger device. The essential oil was analyzed by GC/MS. Trophozoite of T. vaginalis was cultured in vitro in CPLM medium and the effect of the essential oil on the survival of T. vaginalis trophozoite was measured by Neobar slide. This study indicated that Linalool (71.2%) was the most constituent found in C. sativum essential oil. Also, the results of anti-parasitic tests demonstrated the concentrations of 0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003, and 0.001 g/ml in essential oil and 0.25 g/ml in metronidazole could destroy of T. vaginalis trophozoite completely after 420 minutes incubation. The best results were observed at 0.5 and 0.25 g/ml concentrations of essential oil, because these concentrations were able to destroy trophozoite in 90 minutes. Also, 0.001 g/ml concentration of essential oil had the lower anti-parasitic effect than all concentrations against T. vaginalis trophozoite. The trophozoite survived at DMSO after 600 minutes. MIC and MLC of C. sativum essential oil were 0.015 and 0.031 g/ml concentrations, respectively. In our study, the essential oil of C. sativum leaf in several concentrations destroyed T. vaginalis trophozoite. It appears that C. sativum can be used for the treatment of some T. vaginalis infections as an antibiotic.

INTRODUCTION
Antibiotics as one of the most important drugs in pharmacopeia every country, make the fundamental basis for the prevention, control, and treatment of microbial diseases. However excessive use of antibiotics has led to the production of multi-drug resistant strains [1]. One way to limit the resistance of pathogenic microorganism species to the antibiotic is using of plants [2]. Herbal remedies have significant benefits such as fewer side effects, better patient tolerance, and better accessibility [3-5]. Plants as a rich source of ethno medicinal compounds have continued to play a distinguished role in the maintenance of human health against several diseases [6]. Many plants are used for their anti-parasitic prop...
properties [7-9]. Furthermore, the recent development of methods such as essential oil extraction, has increased the interest of herbs researchers to the phytochemical researches [10-13]. Essential oils could be extracted from several parts like roots, stems, leaves, flowers, and fruits. In recent years, interest in essential oils has been incremented for pharmacological studies and it appears that the essential oils have been useful for control and inhibition of animal and human parasitic disease such as Trichomonas vaginalis infections [14-16].

Iran has a rich flora that is widely distributed throughout the country, particularly in the west of Iran [17-19]. In Iranian traditional medicine, herbal medicines have been the basis of treatment and cure for T. vaginalis diseases. A list of medicinal plants in Iran that are consumed for their anti-trichomoniasis properties including Artemisia aucheri Boiss, Zataria multiflora Boiss, Myrtus communis, Allium sativum, Ferula assa-foetida, Lavandula angustifolia, Eucalyptus camaldulensis, Stachys sylvatica, Achillea millefolium, Artemisia absinthium, Juglas regia, Tanacetum parthenium, Taxus baccata, Viola odorata, Pelargonium roseum, Verbas- cum Thapsus, Allium Cepa, Oliveria Decumbens Vent, Muscari neglectum [20].

One of the most important herbal medicines, which is widely consumed in Iranian traditional medicine for the treatment of parasitic infections is Coriandrum sativum or Coriander from Apiales order, Apiaceae family, Coriandrum genus [21]. C. sativum is one of the edible plants which have generated a lot of interest through human history as a medicinal plant. Several extracts of the plant are traditionally used in treating the gastric ulcer, viral, fungal, and bacterial diseases [22]. As far as we know, there is a very little data about the anti-trichomoniasis effect of C. sativum essential oil collected from Kermanshah province, west of Iran. Hence, the aim of the present study was assessment chemical composition and anti-parasitic property of C. sativum on T. vaginalis trophozoite.

**MATERIALS AND METHODS**

**Cultivation of T. vaginalis trophozoite**

T. vaginalis trophozoite was procured from Iran Pasteur Institute. For the mass cultivation, in sterile conditions, we cultured the parasite samples CPLM (Hi-Media Laboratories PVT Ltd Company) at a temperature of 37 °C. Then, we have added 5 ml of the medium to the Coriandrum sativum culture medium and have put at a temperature of 22-24 °C. After 72 hours, we review the medium regarding parasite growth and the absence of bacterial and fungal contamination we repeated this every 72 hours until the intended parasite volume was obtained.

**Plant sample collection and essential oil extraction**

In the tentative study, the plant collected from Kermanshah. The sample was purified from any strange, plants, dust, or any other contaminants. Essential oil from C. sativum leaf extracted by hydro-steam distillation using the Clevenger device was collected and stored in vials. Briefly, 100 to 150 g of the plant was introduced in the distillation flask (1L), which was conjunct to a steam generator via a glass conduit and to a condenser to regain the essential oil. This was retrieved in a funnel spout. Aromatic molecules of the essential oil were assoiled from the plant material and evaporated into hot steam. The hot steam forced the plant material to liberate the essential oil without burning the plant material itself. Then, hot steam containing the essential oil was elapsed through a cooling system to compress the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was cleaned up through anhydrous Na2SO4 to desiccate the yielded essential oil. Then, the essential oil was collected in tightened vials and stored in a refrigerator.

Essential oil of C. sativum analyzed by GC-MS, fused silica DB-5 column with 0.25 μm thickness film was used. The oven temperature was kept at 500°C for 10 minutes and then regulated at 50-2800°C for 40 minutes. Helium flow rate was maintained at 2 ml/min, with the split ratio of 1:3. Ionization voltage of GC/MS was run at 70ev. The constituents of essential oil were recognized by GC/MS. NIST standard reference database (AMDIA version 2.70) was used to interpret the mass spectral data.

**The effect of essential oil of C. sativum on T. vaginalis trophozoite**

Different concentrations of essential oil of C. sativum were prepared the 0.5 g/ml from which nine fold serial dilutions (v/v) (0.5, 0.25, 0.125, 0.062, 0.031, 0.015, 0.007, 0.003, and 0.001 g/ml). Metronidazole (0.25g/ml) was used as a positive control and Dimethyl sulfoxide (DMSO) (Merk, Germany) was used as a negative control. Above doses of C. sativum essential oil, metronidazole and DMSO individually added to T. vaginalis trophozoite content tubes. All tubes were examined unknowingly with Neobar slide regarding viability and motility from the beginning of cultivation with 15- minute intervals up to 3 hours and then in 4, 5, 6, 7, 8, 9, 10 hours. To determine minimum inhibitory concentrations (MIC), all dilutions were prepared from the essential oil in culture media. Then, 60 μl of the microbial suspension was added to each dilution. Finally, the inoculated tubes were incubated at 37 °C for 24 h. After incubation, the tubes were examined regarding the turbidity caused by inoculated the growth of the parasite. The minimum dilution of the essential oil with no turbidity (lack of growth) was considered MIC. To determine minimum lethal concentrations (MLC), all growth-free tubes were cultured by agar media. The inoculated media were incubated at 37 °C for 24 h. The plates with the minimum concentration of essential oil and no parasitical survival were considered MLC of that concentration of the essential oil. Anti-parasitic effect of the C. sativum essential oil was tested five times [23].

**Statistical Analysis**

The data were analyzed by SPSS-22 software using one-way ANOVA followed by Duncan test. To determine the normality of data, the Kolmogorov-Smirnov test was ap-
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RESULTS

Chemical composition of C. sativum essential oil

Overall, fifteen compounds such as α-pinene, β-pinene, p-cymene, limonene, γ-terpinene, linalool, terpinen-4-ole, decanal, nerol, carvacrol, thymol, neryl acetate, 2E-dodecanal, tetrahydro ionol, n-hexadecane were identified in the essential oil of C. sativum using GC/MS (Table 1) and Linalool (71.2%) was the most detected compounds.

In vitro anti-parasitic property of C. sativum essential oil against T. vaginalis trophozoite

In the review of the effect of essential oil of C. sativum on T. vaginalis trophozoite compared with the control groups, it was identified that essential oil of C. sativum at all concentrations (especially at 0.5 and 0.25 g/ml concentrations) destroyed trophozoite in a dose-dependent manner. At the beginning of the experiment, the percent of live trophozoite of DMSO, metronidazole and all concentrations of essential oil was 100% (Fig. 1). Over time, the percentage of trophozoite survival in treated groups with several doses of essential oil and metronidazole decreased. The percent of live trophozoite of the essential oil was 0% in the 0.5 and 0.25 g/ml concentrations after 90 minutes of incubation. While the trophozoite alive at other concentrations of essential oil and metronidazole (Fig. 2). The 0.125 and 0.062 g/ml concentrations of essential oil had 0% of percent of live trophozoite after 135 minutes of incubation, but 0.031, 0.015, and 0.007 g/ml concentrations had 0% of percent of live trophozoite after 180 minutes of incubation.

Table 1. Chemical composition of essential oils of C. sativum

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention Index</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>α- Pinene</td>
<td>939</td>
<td>3.1</td>
</tr>
<tr>
<td>β- Pinene</td>
<td>978</td>
<td>0.4</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1018</td>
<td>2.3</td>
</tr>
<tr>
<td>Limonene</td>
<td>1026</td>
<td>0.3</td>
</tr>
<tr>
<td>γ- Terpinene</td>
<td>1052</td>
<td>8.9</td>
</tr>
<tr>
<td>Linalool</td>
<td>1085</td>
<td>71.2</td>
</tr>
<tr>
<td>Terpinen-4-ole</td>
<td>1164</td>
<td>0.1</td>
</tr>
<tr>
<td>Decanal</td>
<td>1180</td>
<td>0.3</td>
</tr>
<tr>
<td>Nerol</td>
<td>1212</td>
<td>0.6</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>1265</td>
<td>0.3</td>
</tr>
<tr>
<td>Thymol</td>
<td>1276</td>
<td>0.2</td>
</tr>
<tr>
<td>Neryl acetate</td>
<td>1342</td>
<td>7.2</td>
</tr>
<tr>
<td>2E-Dodecanal</td>
<td>1408</td>
<td>3.5</td>
</tr>
<tr>
<td>Tetrahydro ionol</td>
<td>1539</td>
<td>0.4</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>1601</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>99.2</td>
</tr>
</tbody>
</table>

Figure 1. Effect of essential oil of C. sativum on the percent of live T. vaginalis trophozoites in 0, 15, 30, 45 and 60 minutes. Non-identical letters indicate a significant difference between the groups (p ≤ 0.05).
0.015, 0.007, 0.003, 0.001 g/ml concentrations of essential oil and metronidazole showed 12, 24, 27, 41, 58, and 11% of percent of live trichomonad after 135 minutes (Fig. 3). The 0.031, 0.015, 0.007, 0.003 and 0.001 g/ml concentrations of the C. sativum essential oil showed 100% death of the trophozoite after 180, 240, 300, 360 and 420 minutes of incubation, respectively (Fig. 3, 4). While metronidazole at the 0.25 g/ml concentration destroyed the trophozoite completely after 240 minutes of incubation. No anti-parasitic effect was observed due to DMSO during the experiment.

**MIC and MLC determination of C. sativum essential oil**

In the examined parasite, C. sativum essential oil with 0.015 g/ml concentration inhibited T. vaginalis trophozoite growth (MIC) and with 0.031 g/ml concentration destroyed it (MLC) (Table 2).
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Yield and analysis of essential oil of C. sativum

The essential oil yield of the C. sativum essential oil was 0.63%, calculated on the fresh plant. Analysis of the obtained essential oil from the leaf of C. sativum by GC-MS leads to the identification of fifteen components, representing 99.2% of the total essential oil (Table 1). Regarding the chemical constituents, their relative percentage of the total chromatogram area and retention index as it had been shown in table 1. α-pinene, β-pinene, p-cymene, limonene, γ-terpinene, linalool, terpinen-4-ole, decanal, nerol, carvacrol, thymol, neryl acetate, 2e-dodecanal, tetrahydro ionol, n-hexadecane were identified in the essential oil of C. sativum. The main component of C. sativum was linalool (71.2%). Linalool as the main component in C. sativum refers to two enantiomers of a naturally occurring terpene alcohol found in several aromatic plants such as Lavandula, Cinnamomum tamala, Cannabis sativa, Cannabis indica, Ocimum basilicum, Solidago Meyen, Solidago chilensis, Artemisia vulgaris, and Hamulus lupulus [26-29]. The composition of the essential oil of C. sativum in some of the world has been studied and found differ from each other. In a study indicated that essential oil of C. sativum contained 44 compounds mostly of aromatic acids containing capric acid, 2-decenolic acid, undecyl alcohol, undecanolic acid, tridecanolic acid and E-11-tetradecenoic acid and as major constituents [30]. In other study demonstrated essential oil of C. sativum is rich of methyl heptenol, elemol, caryophyllene oxide, geranyl acetate, linanlyl acetate, thymol, geraniol, citronellol, β-caryophyllene, borneol, eucalyptol, β-phellandrene, limonene, and α-pine [31]. In agreement with our results, it is reported that the essential oil of C. sativum contains linalool.

Table 2. MIC and MLC of essential oils of C. sativum (p< 0.01)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>T. vaginalis trophozoite</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (g/ml)</td>
<td>0.015</td>
</tr>
<tr>
<td>MLC (g/ml)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Figure 4. Effect of essential oil of C. sativum on the percent of live T. vaginalis trophozoites in 240, 300, 360, 420, 480, 540, and 600 minutes.

Non-identical letters indicate a significant difference between the groups (p ≤ 0.05).

DISCUSSION

Volvo vaginitis as a usual medical problem can lead to remarkable inconvenience and repeated medical visits. Infection, allergy, and systemic diseases can happen due to Volvo vaginitis. Its main causes before menopause are bacterial vaginitis, Volvo vaginal candidiasis, and T. vaginalis [24]. T. vaginalis causes vaginitis in women and rarely urethritis and sometimes prostatitis in men. The symptoms either do not or rarely appear in men and starts with discharge and itching in women. Given the importance of the disease and the problems of controlling and combating due to sexual transmission, the drug resistance and the lack of appropriate and certain vaccines, a need is felt for appropriate, safe, inexpensive and accessible medical approaches [25]. One way to prevent and control the resistance of pathogenic microorganisms to the antibiotic is using ethno medicinal plants. C. sativum as a plant in Iranian traditional medicine has been indicated to have some optimal treatment properties, due to its antioxidant and anti-inflammatory effects in both in vitro and in vivo [21,22]. But, to our knowledge, this is the first time essential oil of C. sativum collected in Kermanshah has been used for its anti-trichomoniasis effects.

The essential oil yield of the C. sativum essential oil was 0.63%, calculated on the fresh plant. Analysis of the obtained essential oil from the leaf of C. sativum by GC-MS leads to the identification of fifteen components, representing 99.2% of the total essential oil (Table 1). Regarding the chemical constituents, their relative percentage of the total chromatogram area and retention index as it had been shown in table 1. α-pinene, β-pinene, p-cymene, limonene, γ-terpinene, linalool, terpinen-4-ole, decanal, nerol, carvacrol, thymol, neryl acetate, 2e-dodecanal, tetrahydro ionol, n-hexadecane were identified in the essential oil of C. sativum. The main component of C. sativum was linalool (71.2%). Linalool as the main component in C. sativum refers to two enantiomers of a naturally occurring terpene alcohol found in several aromatic plants such as Lavandula, Cinnamomum tamala, Cannabis sativa, Cannabis indica, Ocimum basilicum, Solidago Meyen, Solidago chilensis, Artemisia vulgaris, and Hamulus lupulus [26-29]. The composition of the essential oil of C. sativum in some of the world has been studied and found differ from each other. In a study indicated that essential oil of C. sativum contained 44 compounds mostly of aromatic acids containing capric acid, 2-decenolic acid, undecyl alcohol, undecanolic acid, tridecanolic acid and E-11-tetradecenoic acid and as major constituents [30]. In other study demonstrated essential oil of C. sativum is rich of methyl heptenol, elemol, caryophyllene oxide, geranyl acetate, linanlyl acetate, thymol, geraniol, citronellol, β-caryophyllene, borneol, eucalyptol, β-phellandrene, limonene, and α-pine [31]. In agreement with our results, it is reported that the essential oil of C. sativum contains linalool.

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<tr>
<td>MIC (g/ml)</td>
<td>0.015</td>
</tr>
<tr>
<td>MLC (g/ml)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Figure 4. Effect of essential oil of C. sativum on the percent of live T. vaginalis trophozoites in 240, 300, 360, 420, 480, 540, and 600 minutes.

Non-identical letters indicate a significant difference between the groups (p ≤ 0.05).

Table 2. MIC and MLC of essential oils of C. sativum (p< 0.01)

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<td>0.031</td>
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</table>
(60-70%) [32]. Also in other studies revealed linalool as major constituents of essential oil of C. sativum [33-35].

Anti-trichomoniasis property of essential oil of C. sativum

In this study, the anti-parasitic properties of C. sativum essential oil on T. vaginalis trophozoite were assessed. The result indicated that after 420 minutes of incubation of the parasite with several concentrations of essential oil had 100% death of trophozoite. According to the results, the essential oil demonstrated the highest anti-parasitic effect on trophozoite at 0.5 and 0.25 g/ml concentrations. Another main characteristic of our study was that C. sativum essential oil with 0.015 g/ml concentration inhibits T. vaginalis trophozoite growth and with 0.031 g/ml concentration destroyed it. There are several studies about the antiparasitic effect of C. sativum, but there isn’t study about the anti-trichomoniasis effect of it [36-38]. In studies indicated C. sativum have antiparasitic effects against Tribolium confusum and Callosobrachus maculatus [36, 37]. Also in other study demonstrated the anthelmintic property of C. sativum against Pheretima posthumada [38]. Indeed, the anti-parasitic effect of the C. sativum is related to its constituent constituents. In our study, the main compound in the essential oil was linalool. In studies indicated linalool have good antibacterial and antiparasitic effect and can use as the antimicrobial supplement or drug [39-41].

CONCLUSION

In this study, essential oil of C. sativum as an aromatic medicinal plant possess anti-trichomoniasis effect against T. vaginalis trophozoite. The trophozoite was destroyed completely by the several doses of examined essential oil (The best results were observed at 0.5 and 0.25 g/ml concentrations of essential oil). Also, the results indicate that essential oil of C. sativum has its chemical composition, which is attributed to its anti-trichomoniasis activity. These compounds (especially linalool) can be used as anti-trichomoniasis supplement or drug. Fractionation and characterization of active molecules will be the future work to investigate.

ACKNOWLEDGMENT

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CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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