Inhibitory effect of Carum carvi essential oil on the growth of Candida albicans

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ABSTRACT

Candida albicans is the opportunistic yeast which causes serious health problems in human and animals. Resistance of C. albicans against many commercial drugs has made it necessary to explore natural resources for finding new powerful antifungal agents. In the present study, effect of different concentrations of Carum carvi essential oil (0.015-4%, v/v) on growth of C. albicans were investigated by microbroth dilution assay in 96 well microplate. The inhibitory effect of C. carvi essential oil was monitored by colony growth rate and spectrophotometrically at the 520 nm wave length. Results showed that the plant essential oil strongly inhibited growth of C. albicans dose-dependently. C. carvi oil at the concentration of 0.5 and 1% caused the reduction in CFU as 50% (MIC\textsubscript{50}) and 90% (MIC\textsubscript{90}) of control, respectively. Essential oil of C. carvi at concentration of 2% completely inhibited formation of fungal colonies and could be considered as a potential candidate for treatment of C. albicans related diseases.

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1. Introduction

The blastomycete opportunistic yeast, Candida albicans invades different areas of the human body causing cutaneous, mucocutaneous and opportunistic infections. Infections caused by C. albicans have been among the most common microbial infections all over the world (Terai et al., 2010). Candida albicans causes opportunistic
infections such as oral candidiasis, oesophageal candidiasis and vaginal candidiasis. It is known that oral and oesophageal candidiasis cause oral cavity pain, tongue pain, taste disturbance, odynophagia or dysphagia (Runyoro et al., 2006). Since symptoms of oral and oesophageal candidiasis appear in oral regions, eating function is greatly deteriorated. The reduction of food intake leads to under-nutrition and impaired quality of life, and sometimes causes life-threatening risks. Regarding limitations of synthetic antifungal drugs such as high production cost and numerous side effects together with acquired resistance of Candida species, plants provide potential resources for production of antimicrobial and antifungal medicines. Antibacterial and antifungal effects of many plant species have been reported (Van Vuuren et al., 2009). Different parts of plants including leaves, fruits and even seeds can be used as potential antifungal or antibacterial material (Verma, 2007). Essential oils constitute a vast majority of natural antifungal materials. They are composed of different ingredients with high power of inhibiting or even eliminating various pathogens (Razzaghi-Abyaneh et al., 2008 and 2011).

Carum carvi (Black Zira, Caraway) is an endemic plant of Iran which belongs to Apiaceae family. Containing high concentration of essential oils, Carum carvi is regarded as a powerful antifungal and antimicrobial medicine in both traditional and modern medicine (Razzaghi-Abyaneh et al., 2013). Antimicrobial effects of this herb on many gram positive and gram negative bacteria have been well documented (Grigore et al., 2012).

In the present study, antifungal activity of C. carvi essential oil against a pathogenic strain of C. albicans was evaluated.

2. Materials and methods

Candida albicans PFCC 50271 was obtained from the Pathogenic fungi: Culture Collection of the Pasteur Institute of Iran (www.pasteur.ac.ir). Essential oil of C. carvi was prepared by hydrodistillation as described by Alinezhad et al., 2011. In a microbroth dilution assay, 100 µl of RPMI along with different concentrations of C. carvi oil ranging from 0.015-4% were prepared in 96-well microplate and 1µl of the yeast suspension (10⁶ cells/ml) was added to each well. Broth medium with yeast suspension was used as control. MIC values were determined after 24 h incubation at 35 °C by reading the cultures absorbance at 520 nm using a spectrophotometer and counting the number of colony forming units (CFU) after transferring to Sabouraud dextrose agar plates. The lowest concentration which resulted in 90% reduction of CFU compared to control was assigned as MIC₉₀. Moreover, the lowest concentration that completely inhibited formation of fungal colony was assigned as minimum fungicidal concentration (MFC). To compare efficiency of C. carvi oil with commercial synthetic medicines, various dilutions of the antifungal drug fluconazole were used in a parallel assay.

3. Results

In the present work, C. albicans PFCC 50271 was subjected to microbroth dilution assay. After incubation with serial dilutions of C. carvi essential oil, colony forming units (CFU) of the yeast was evaluated (Fig. 1). As shown in Fig. 1, the plant oil reduced CFU of C. albicans dose-dependently. Detailed data of CFUs and OD of control and treated cultures are summarized in Table 1. C. carvi oil at the concentration of 1% caused the reduction in CFU as 90% of control. So 1% concentration of essential oil was considered as MIC₉₀. Similarly, 0.5% concentration of essential oil resulted in CFU=9000 which is half of CFU of control (18000); therefore 0.5% concentration was regarded as MIC₅₀. Finally, essential oil of C. carvi at concentration of 2% completely inhibited formation of fungal colonies. So it was considered as MFC.

Considering OD values, it can be observed that by reduction of essential oil concentration, OD values of fungal growth were increased. This is due to the fact that in reduced concentration of essential oil, the yeast can grow better which leads to turbidity of the medium and consequently enhancement of OD values.

Table 1

<table>
<thead>
<tr>
<th>OD values and CFU counting of C. albicans exposed to in various concentrations of C. carvi oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>OD</td>
</tr>
<tr>
<td>CFU</td>
</tr>
</tbody>
</table>
As mentioned in materials and methods section, a parallel assay was carried out to investigate the effects of various dilutions of fluconazole (from 2-256 µg/ml) as a commercial antifungal drug. The results of this assay are presented in Table 2. As shown in Table 2, fluconazole at the concentration of 128 µg/ml. The drug at concentration of 8 µg/ml reduced CFU as 50%.

<table>
<thead>
<tr>
<th>OD</th>
<th>CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8500</td>
</tr>
<tr>
<td>4</td>
<td>5000</td>
</tr>
<tr>
<td>8</td>
<td>2600</td>
</tr>
<tr>
<td>16</td>
<td>1500</td>
</tr>
<tr>
<td>32</td>
<td>340</td>
</tr>
<tr>
<td>64</td>
<td>167</td>
</tr>
<tr>
<td>128</td>
<td>0</td>
</tr>
<tr>
<td>256</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>10000</td>
</tr>
</tbody>
</table>

Table 2
OD values and CFU counting of C. albicans exposed to various concentrations of fluconazole (µg/ml).

4. Discussion

The results obtained in the present study indicated that essential oil of C. carvi is a powerful agent to inhibit growth of C. albicans. Begum et al. (2008) investigated the effect of C. carvi essential oil on a wide range of bacteria and fungi and indicated that the oil was able to inhibit growth even at low concentrations. In an investigation conducted by Gholami et al. (2012), it was shown that C. carvi essential oil can inhibit growth of C. glabrata isolates which were resistant to fluconazole. Cosic et al. (2012) investigated effects of essential oils of eleven herbs including C. carvi on different fungi. They showed that C. carvi oil was able to inhibit growth of nine fungi but failed to inhibit the growth of the others. Inhibitory effect of caraway oil on Escherichia coli, Pseudomonas and Candida was also reported by Grigore et al. (2012). Inhibitory effects of C. carvi oil on pathogens other than Candida have also been reported. Siripornvisal et al. (2011) showed that essential oil of C. carvi inhibited growth of Aspergillus, Fusarium and Botrytis. Antimicrobial effects of C. carvi were attributed to presence of compounds such as carvone and limonene (Iacobellis et al., 2005).

As a conclusion, the results obtained in this study reinforce potential antifungal effects of essential oil of C. carvi. Comparing MIC value of C. carvi oil with those of fluconazole as an antifungal drug showed that C. carvi oil at concentration of 2% is as powerful as the concentration of 128 µg/ml of fluconazole. This is a promising result for
potential application of *C. carvi* instead of synthetic antifungal drug. An appropriate formulation of *C. carvi* essential oils can be a good substitute for synthetic drugs. This needs further investigations that can be the subject of our research works in future.

**References**


