Sjournals
Scientific Journals
Scientific Journal of Microbiology (2014) 3(7) 74-77
ISSN 2322-2948
doi: 10.14196/sjm.v3i7.1510

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

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

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ARTICLE INFO

Article history,
Received 23 June 2014
Accepted 18 July 2014
Available online 29 July 2014

Keywords,
Candida albicans
Carum carvi
Essential oils
Inhibitory effect

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 make 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 spectrophotomically 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_{50}) and 90% (MIC_{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.

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, roots 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, *C. 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 (10^6 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 ºC 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 MIC_{90}. 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 MIC_{90}. 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 MIC_{50}. 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 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

OD values and CFU counting of *C. albicans* exposed to in various concentrations of *C. carvi* oil

	0.060	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**Control**
OD	0.033	0.030	0.026	0.020	0.011	0.002	0.001	0.036
CFU	16000	14000	11000	9000	160	0	0	18000

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 show in Table 2, fluconazole at the concentration of 128 µg/ml. The drug at concentration of 8 µg/ml reduced CFU as 50%.

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

	2	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**Control**
OD	0.030	0.029	0.027	0.025	0.023	0.022	0.021	0.013	0.032
CFU	8500	5000	2600	1500	340	167	0	0	10000

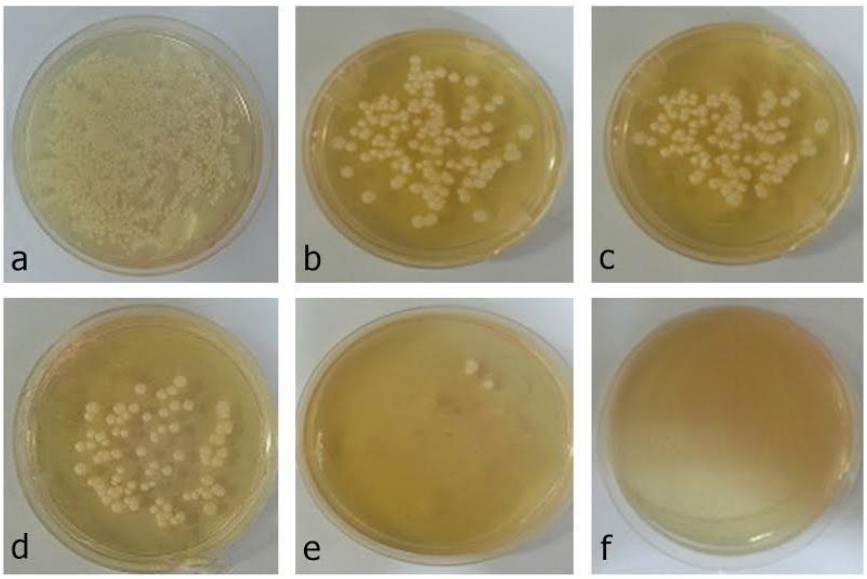

Fig. 1. Dose-dependent growth inhibition of *Candida albicans* by *Carum carvi* essential oil assessed by CFU determination on Sabouraud dextrose agar plates. Control (a) and plant-exposed cultures of 0.125 (b), 0.25 (c), 0.5 (d), 1.0 (e), and 2.0% (f) are shown.

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* 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 rein force 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.

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