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# Chemical composition, antioxidant activity and antifungal effects of five Iranian essential oils against *Candida* strains isolated from urine samples

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ABSTRACT

Systemic candidiasis has become an emerging fungal infection in recent years. Anti-*Candida* resistance to conventional antifungal agents has subsequently increased. This study reported the chemical composition, antioxidant and anti-*Candida* activity of *Origanum majorana*, *Artemisia dracunculus*, *Cymbopogon citrate*, *Cinnamomum verum* and *Caryophyllus aromaticus* essential oils. Different *Candida* species, from urine tracts of hospitalized patients, were included to be challenged with understudied essential oils. Chemical compositions were determined using gas chromatography/mass spectroscopy (GC/MS) analysis and antioxidant activity was measured using DDPH assay. MIC of these essential oils was evaluated using broth micro-dilution test. *Caryophyllus aromaticus* had the highest antioxidant activity while the lowest antioxidant activity was for *Artemisia dracunculus*. MICs of *Cinnamomum verum*, *Caryophillium aromaticus*, *Artemisia dracunculus*, *Origanum vulgare* and *Cymbopogon citratus* essential oils ranged from 125 to 175 μg/mL (mean value: 147.7 ± 25.5 μg/mL), 700 to 1000 μg/mL (mean value: 740.9 ± 105.4 μg/mL), 1000 to 2000 μg/mL (mean value: 1454.5 ± 509.6 μg/mL), 173 to 350 μg/mL (mean value: 208 ± 55.8 μg/mL) and 125 to 175 μg/mL (mean value: 156.8 ± 24.6 μg/mL) for different *Candida* species, respectively. In general, natural compounds are suitable to be used as anti-*Candida* and antioxidant agents. However in this stage, these compounds could be applied as supplementary agents along with conventional antifungal drugs.

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## Introduction

Systemic candidiasis has been the most common invasive fungal infection in recent years [1]. It is caused by different *Candida* species and *Candida albicans* has been the main species isolated from *Candida* infected patients [2,3]. However non-*albicans Candida* species incidence is increasing nowadays [4–6]. *Candida* spp. have been the fourth most prevalent nosocomial pathogen in intensive care units [7]. These species have the potential to develop antifungal resistance either intrinsically or during treatment [5,8–12]. Hence introducing new antifungal agents is difficult due to eukaryotic nature of fungi [13–15]. Interest in application of natural compounds with antifungal activities against fungal pathogens has grown [16–18].

Essential oils come from plant secondary metabolisms [19]. They are composed of mono and sequiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones [19,20]. The antimicrobial activity of essential oils has been well documented. However there are still some contradictory results on their activity. Their antimicrobial is not fully understood yet, however it is believed that having numerous different groups of chemical compounds, several targets must exist in the fungal cells.

This study was designed to evaluate antifungal activity of five Iranian essential oils on *Candida* species isolated from patients' urine samples and its connection to their chemical composition and antioxidant properties.

## Materials and methods

### Test microorganisms

The *Candida* isolates used in the present study were *C. albicans* ($n = 11$), *C. tropicalis* ($n = 4$), *C. krusei* ($n = 2$), *C. glabrata* ($n = 3$), *C. parapsilosis* ($n = 1$) and *C. famata* ($n = 1$), which were obtained

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from urine samples of patients admitted to the intensive care unit in Arad Hospital, Tehran, Iran. The identification of *Candida* species were confirmed by germ tube test, CHROM agar, urease test, sugar fermentation and assimilation tests by RAPID yeast plus system (Remel Inc., Lenexa, KS, USA).

*Isolation and preparation of essential oils*

Leaves of *Origanum majorana*, *Artemisia dracunculus* and *Cymbopogon citrate*, bark of *Cinnamomum verum* and flower buds of *Caryophyllus aromaticus* were purchased from the Pakan Bazr Company in Isfahan, Iran. All plants were taxonomically identified at the Pharmacognosy Department, Faculty of Pharmacy, University of Tehran, Iran.

The plants were submitted to hydrodistillation in a Clevenger-type apparatus at 100 °C for 5 h. The EOs were isolated and dried over anhydrous sodium sulfate and then stored in a dark glass bottle at 4 °C until required.

*Components separation and identification of essential oils by gas chromatography/mass spectroscopy (GC/MS) analysis*

The extracted compounds were analyzed on a 6890 N Agilent gas chromatograph coupled to a 5975 C Agilent mass-selective detector (Agilent Technologies, Avondale, PA, USA) with a 7683 Agilent auto sampler and 1.0 μL of the sample were injected in the split less mode at 250 °C into a 30 m × 0.25 mm × 0.5 μm DB-5 MS capillary column and operated by MSD Chemstation Software (Agilent Technologies).

The temperature program used for the chromatographic separation was as follows: 50 °C for 2 min, temperature increase at 25 °C·min$^{-1}$ to 100 °C and hold for 2 min, then temperature increase at 5 °C·min$^{-1}$ to 290 °C where it was finally held for 5 min. The carrier gas was helium (99.999%) and was kept at a constant flux of 1.0 mL·min$^{-1}$. The mass spectrometer was operated in the electron impact ionization mode and the energy of the electrons was kept at 70 eV. After injection of sample to GC/MS several unknown peaks were observed. The impact mass spectra of these obtained peaks were searched for in our computer library.

*Antifungal susceptibility assays*

*Disk diffusion*

The *Candida* isolates were tested by disk diffusion method as described in the document M44-A from the Clinical Laboratory Standards Institute (CLSI) for yeasts using Muller-Hinton agar supplemented with 2% glucose and 0.5 g/mL of methylene blue. The agar surfaces were inoculated by using a swab dipped in a yeast cell suspension adjusted to ($10^6$ cells/mL). The standard filter paper discs of fluconazole (25 μg/mL), itraconazole (10 μg/mL) and voriconazole (1 μg/mL) (Becton Dickinson, Sparks, MD, USA) were placed on the inoculated plates and were incubated at 37 °C for 48 h. Inhibition zones (mm) were interpreted using validated CLSI interpretive break points for these drugs. For quality control, *C. albicans* ATCC 10241 and *C. krusei* ATCC 6258 were used. Each experiment was tested in duplicate.

*Determination of minimum inhibitory and fungicidal concentration*

Susceptibility of *Candida* isolates to fluconazole, itraconazole, voriconazol and studied EOs was done by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) M27-A3 document. Briefly, different concentration of EOs (15.6, 31.2, 62.5, 125, 175,250, 350, 500, 700, 1000 and 2000 μg/mL) and antifungals (0.031–64 μg/mL) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, USA) buffered with MOPS (Sigma, St. Louis, MO, USA). Working inoculums were adjusted density of $1 \times 10^3$ CFU/mL with haemocytometer (0.1 mL) was added to the Microtiter plates, which were incubated at 30 °C for 24–48 h. Non-inoculated medium (200 mL) was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without essential oil or/and antifungals) were also included. The growth in each well was compared with that of the growth in the control well.

MICs were visually determined and defined as the lowest concentration of the essential oil produced growth inhibition compared with the growth in the control well. Aliquots of the mixture of oil and the *Candida* suspension, which showed negative-visible growth after the first 24 h of incubation, were inoculated onto the surface of the Sabouraud dextrose agar. The lowest concentration of the oil giving negative growth of the *Candida* was recorded as the minimum fungicidal concentration (MFC). All experiments were repeated on three separate occasions, with triplicate determinations on each occasion.

*Antioxidant activity*

*2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay*

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). Briefly, 50 mL of various concentrations of the EOs were added to 5 mL of the DPPH solution (0.004% methanol solution). After 30 min of incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation:

$I\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100$

Where $A_{blank}$ is the absorbance of the control (containing all reagents except the test compound) and $A_{sample}$ is the absorbance of the test compound. EO concentration providing 50% inhibition (IC50) was calculated from the linear regression algorithm of the graph plotted inhibition percentage against the EO concentration using PHARM/PCS Version 4 (Springer-Verlag New York Inc., New York). Tests were carried out in triplicate.

*Statistical analysis*

Data were subjected to analysis of variance (One-way Anova) test. Mean comparisons were performed using the high significant difference (HSD) Tukey's test to examine if differences were significant at $P < 0.05$. All analyses were performed with statistical products and service solutions (SPSS) software package v. 17.0 for Windows (Microsoft Corporation, USA).

## Results and discussion

In recent years, new researches about biological active compounds present in the oils of the plants have been seen as a potential way to control fungal contamination and interest in the application of these complex mixtures in the treatment of microbial diseases has notably increased. In current study, the anti-*Candida* and antioxidant activity of oils from five medicinal plants that had been used in local folk medicinal remedies in different forms for various afflictions in Iran was evaluated (List of the plants and their properties are given in Table 1).

Twenty-four, eleven, thirty-five, seventeen and fifteen components comprising over 98.57, 99.73, 97.93, 99.06 and 99.01% were identified in *Cinnamomum verum*, *Caryophillium aromaticus*, *Artemisia dracunculus*, *Origanum vulgare* and *Cymbopogon citratus*

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