In Vitro Comparison of the Efficacy of Cumin Extract and Fluconazole Against Candida Strains

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Abstract
Background and Aim: Fungal infections are the most common opportunistic infections; when accompanied by widespread infections, such as acquired immune deficiency syndrome (AIDS), they cause a worldwide health crisis. Therefore, treatment of these infections is seen as one of the health challenges. Medicinal herbs can be used as rich sources of treatment due to their fewer side effects. Currently, Cuminum cyminum (cumin) is considered to have antimicrobial properties. The aim of this study was to compare the efficacy of cumin extract and fluconazole against Candida species in vitro.

Materials and Methods: In this in-vitro experimental study, aqueous and ethanolic extracts of cumin were prepared through maceration. The minimum inhibitory concentrations (MIC) of aqueous and alcoholic extracts of cumin against Candida albicans and Candida glabrata were determined using the microdilution method. Fluconazole was simultaneously used as an antifungal agent for comparison.

Results: Alcoholic and aqueous extracts of cumin were able to prevent the growth of Candida albicans at MICs of 100 mg/ml and 200 mg/ml, respectively. Both aqueous and alcoholic extracts of cumin were able to prevent the growth of Candida glabrata at 200 mg/ml concentration. Fluconazole was able to inhibit the growth of Candida albicans and Candida glabrata at MICs of 0.12 mg/ml and 0.03 mg/ml, respectively.

Conclusion: According to the findings of this study, both aqueous and alcoholic extracts of cumin exhibited measurable inhibitory activities against Candida species.

Key Words: Cuminum cyminum, Candida albicans, Candida glabrata, Fluconazole

Introduction
Invasive fungal infections have been increasing since a few decades ago. These infections increase mortality rates as opportunistic lesions in the oropharyngeal region in patients with an impaired immune system (HIV/AIDS). The
results of epidemiological studies show that Candida infections are the fourth most common nosocomial infections [1]. *Candida albicans* is a major pathogen; however, other Candida strains, such as *Candida glabrata*, *Candida krusei*, and *Candida tropicalis*, are also pathogenic and have been isolated from patients. The importance of species other than *Candida albicans* has increased in recent years due to relative resistance of some of these species (e.g. *Candida glabrata* and *Candida tropicalis*) to some antifungal drugs. *Candida albicans* is a fungal microorganism found as normal flora in the human mouth and digestive system, which turns into a pathogen as the immune system weakens [2]. *Candida glabrata* is found as a saprophyte in the oral mucosa; 31-55% of this species have been isolated from the oral cavity of healthy people. The resistant form of this species is found in cancer patients and develops lethal lesions [3,4]. Biofilm formation in urinary catheters and on dental equipment and prostheses is a pathogenic feature of this fungus [5].

Repeated administration of antifungal agents can develop resistance of these microorganisms to antifungal drugs, which is considered as a major barrier against treatment. The limited number, high costs, and extensive side effects of these drugs are barriers against the effective treatment of progressive fungal infections [6]. Fluconazole is known as one of the most effective antifungal drugs that belong to the Azole group. Oral and injection routes of administration are available for this drug. It can be used to treat Candidemia or Candida infection disseminated to the bloodstream. This drug has many disadvantages, including drug interaction with warfarin, ingestion through the gastrointestinal route, and drug resistance [7]. *Cuminum Cuminum* (Cumin) is an effective herb in the treatment of fungal infections. It is also aromatic and is used to inhibit fungal growth, to prevent aflatoxin contamination, and to help store food (wheat and pea). In addition to the food industry, it is used to treat gastrointestinal disorders, such as diarrhea, epilepsy, and spasm. It belongs to the Umbelliferae family. It was used as an antispasmodic drug in earlier times since it contains cumin aldehyde, limonene, α-cymene, β-pinene, terpinene, and carvone [8].

Due to the increasing acquired resistance of Candida strains to antifungal agents, it is inevitable to synthesize new compounds with antifungal features and minimum side effects to combat fungal diseases as alternatives to chemical drugs with several side effects, causing microbial resistance and having expensive production processes in comparison with herbal medications. The antimicrobial properties of some herbs are attributed to compounds such as polyphenols [8]. The medicinal properties of this plant vary based on the geographical location. Therefore, the aim of the present study was to examine the effect of the Iranian type of this plant in comparison with fluconazole as a standard medication in the treatment of infection with *Candida albicans* and *Candida glabrata*.

**Materials and Methods**

This was a laboratory-based research with an experimental intervention. Cumin samples were purchased from a traditional pharmacy store (not a licensed drug store but rather a store where herbal remedies for various diseases are sold) by a botanist as a member of the School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. This plant was collected from Khorasan province in late autumn in 2013. Studied pathogens were standard strains of *Candida glabrata* and *Candida albicans* prepared from the collection of the Iranian Institute for Applied and Industrial Research. To determine the smallest inhibitory zone, eight concentrations of aqueous and alcoholic extracts of cumin and fluconazole were selected to test *Candida albicans* and *Candida glabrata*. Given that these microbiological tests are repeated three times in standard trials, each strain was tested three times at the given concentration of the drug. Three plates (aqueous extract, alcoholic extract, and fluconazole) were prepared for each strain. In total, six plates were prepared for the two strains.
Aqueous and alcoholic extracts of cumin were prepared through maceration which is one of the herbal extraction methods.

*Preparation of the aqueous extract:*
One-hundred grams of dried cumin was weighed using a digital scale and powdered using an electric mill. The powder was poured into an Erlenmeyer flask, and 1000 ml of distilled water was added to the powder which was warmed up in a heater for 10 minutes. The Becher cap was coated with aluminum foil for 40 hours. The suspension was filtered using a filter paper. An aqueous extract at 10% concentration was prepared using a water bath (Figure 1) [9].

![Figure 1. Aqueous and alcoholic extracts of cumin at 10% concentration](image)

*Preparation of the alcoholic extract:*
One-hundred grams of cumin powder was prepared as described in the previous section, and 1000 ml of 96% ethanol was added to the powder, completely covering the powder in the container. The Erlenmeyer flash cap was coated with an aluminum sheet. The flash was shaken using a shaker device at 90 rpm (revolutions per minute) for 48 hours. The solvent and the herb were completely mixed to reach a homogeneous solution. The solution was filtered using a filter paper. The solution was evaporated using a rotary evaporator (vacuum distillation) to remove the solvent from the extract. An alcoholic extract at 10% concentration was prepared, which was kept in a refrigerator before the microbial tests (Figure 1) [9].

*Selecting the proper yeast species:*
The samples were prepared from the Persian Type Culture Collection (PTCC) and included *Candida glabrata* (PTCC5295) and *Candida albicans* (PTCC5027). All of the samples were collected from the collection of the Iranian Institute of Applied and Industrial Research. To determine the antifungal properties in this study, fluconazole powder with 98% potency (serial number: 044K258, Sigma-Aldrich Corp., Germany) was selected.

*Preparation of Mueller-Hinton broth:*
To prepare Mueller-Hinton broth (Merck KGaA, Darmstadt, Germany), 2.1 g of the powdered medium was added to 100 cc of distilled water or ion-free water. The medium was heated to completely dissolve the powder in water. The solution was sterilized in an autoclave at 121°C for 15 minutes. The solution was cooled down and used for microdilution in microplates.

*Preparation of Mueller-Hinton agar:*
To prepare Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany), 3.4 g of the powdered medium was added to 100 cc of distilled water or ion-free water. The medium was heated to completely dissolve the powder in water. The solution was sterilized in an autoclave, cooled down, and divided in microplates.

*Preparation of 0.5 McFarland turbidity:*
First, 1% sulfuric acid was prepared from 98% sulfuric acid. Then, 99 cc of distilled water was mixed with 98% sulfuric acid (water should be added since the reaction between the acid and water is exothermic). Then, 1.175 g of barium chloride powder was added to 100 cc of distilled water to prepare 1.175% barium chloride. Then, 99.5 cc of 1% sulfuric acid was mixed with 0.5 cc of barium chloride. If barite deposition (barium sulfate, BaSO₄) was found as an opaque shape in the tube, the opacity would be considered as 0.5 McFarland standard. Each microbial suspension contained 1.5×10⁸ colony-forming units per milliliter (CFU/ml). The comparison was made through bare eyes in the light. The light absorbance of a 0.5 McFarland suspension is 0.08-0.1 at a 625-nm wavelength [10].

*Microbial culture in suitable media:*
The Candida strains were cultured on Mueller-
Hinton agar using sterilized needles. The plate was incubated at 37°C for 24 hours. After this period, Candida colonies grew in the medium (Figure 2).

Preparation of microbial suspensions:
Mueller-Hinton agar was prepared at 37°C for 24 hours to culture Candida albicans and Candida glabrata. Fungal specimens were prepared in sterile physiological suspension serum. The 0.5 McFarland standard (1.5×10⁸ CFU/ml) was used to obtain uniform or homogeneous suspensions with similar concentrations of fungal specimens.

Preparation of a series of dilutions from aqueous cumin extract:

![Figure 2. Candida albicans and Candida glabrata cultured on Mueller-Hinton agar](image)

Hypothetical dilutions were selected to include a wide range of different concentrations of the fungus (1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 mg/ml). To prepare these dilutions, eight sterile microplates containing 100 μl of Mueller-Hinton broth culture medium were prepared. Then, the cumin extract was diluted to 400 mg/ml with 2% dimethyl sulfoxide (DMSO) solution, and 100 μl of it was added to the first well. From the dilution prepared in the first well, we removed 100 μl and added it to the second well; thus, by transferring 100 μl from each well to the next well and dispensing 100 μl of the final tube, serial dilutions were provided. This experiment was repeated three times.

Exposure of the microbial suspensions to different dilutions of cumin extract:
The fungal suspension was diluted (1:20) with distilled water, and then, 10 μl of the fungal suspension was added to the wells and incubated at 37°C for 24 hours. Finally, the microbial suspension was at a concentration of 5×10⁴ CFU/ml in each well.

Microdilution according to the principles of the Clinical and Laboratory Standards Institute (CLSI) [11]:
Since the color of the extract’s opacity is not visible to the eye, the opacity should be read by reading an optical density (OD) on an ELISA reader or the growth in each well with sterile anise in Mueller-Hinton Agar culture medium; therefore, the second method was considered. For this reason, the media were again incubated at 37°C. After 24 hours, the presence or absence of a colony was examined with the bare eye and compared with positive and negative control plates.

Mueller-Hinton culture medium was considered as a positive control, while Candida medium was considered as a negative control.

Determining the minimum inhibitory concentration (MIC):
A well with a minimum concentration of the extract that had inhibited fungal growth and contained few fungal colonies was considered as the MIC. As expected, no fungal growth was found with the next concentration [11]. The time required by the minimum concentration of the extract to be effective against the fungi was 24 hours; therefore, the effective time was considered to be 24 hours [10].

Dilution and determination of the effect of alcoholic cumin extract:
Dilution and determination of the effect of alcoholic cumin extract on the two species of Candida were performed according to the protocol used for the aqueous extract.

Preparation of fluconazole dilution series:
The antifungal effects of aqueous and alcoholic extracts of cumin and fluconazole were compared. The Sigma protocol was used to prepare the fluconazole dilution series. One cc of 2% DMSO was added to 1.024 mg of fluconazole powder to obtain 1.024 mg/ml concentration of fluconazole. Other dilutions series were prepared according to the above protocol.

Data analysis:
The results of the study were presented as
Results
In this study, the antifungal effects of aqueous and alcoholic extracts of cumin in 2% DMSO solvent at different concentrations on the growth of Candida albicans and Candida glabrata were studied by the microdilution method to determine the MIC with three repeats.

Candida Albicans:
Contents of Table 1 show that alcoholic extract of cumin at a concentration of 100 mg/ml and aqueous extract of cumin at a concentration of 200 mg/ml inhibited the growth of Candida albicans (Figures 3 and 4).

In comparison, fluconazole at a concentration of 0.20 mg/ml inhibited the growth of Candida albicans (Table 2 and Figure 5).

Candida glabrata:
Contents of Table 1 show that aqueous and alcoholic extracts of cumin at a concentration of 200 mg/ml inhibited the growth of Candida glabrata (Figures 3 and 4).

In comparison, fluconazole inhibited the growth of Candida glabrata at a concentration of 0.03 mg/ml (Table 2 and Figure 5).

The MICs of aqueous and alcoholic cumin extracts were different than the MIC of fluconazole for each strain (Figures 3 to 6). Similar MICs were obtained in each of the three repeats for each strain and with each extract. Since the MIC was a constant value in each of the three repeats for each strain and with each extract, it is not possible to compare the data and perform statistical tests between the groups.

Table 1. Minimum inhibitory concentration (MIC) of alcoholic and aqueous extracts of cumin against the growth of Candida albicans and Candida glabrata

| Row | Concentrations (mg/ml) | Candida albicans | Candida glabrata |
|-----|------------------------|------------------|------------------|
|     |                        | Aqueous          | Alcoholic        | Aqueous | Alcoholic |
| 1   | 200                    | -                | -                | -       | -         |
| 2   | 100                    | +                | -                | +       | +         |
| 3   | 50                     | +                | +                | +       | +         |
| 4   | 25                     | +                | +                | +       | +         |
| 5   | 12.5                   | +                | +                | +       | +         |
| 6   | 6.25                   | +                | +                | +       | +         |
| 7   | 3.125                  | +                | +                | +       | +         |
| 8   | 1.562                  | +                | +                | +       | +         |

+: fungal growth  
- : inhibited growth

Figure 3. Evaluation of the minimum inhibitory concentration (MIC) of aqueous extract of cumin against the growth of Candida albicans at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 mg/ml in three phases of repetition of the experiment.
**Figure 4.** Evaluation of the minimum inhibitory concentration (MIC) of alcoholic extract of cumin against the growth of *Candida albicans* at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 mg/ml in three phases of repetition of the experiment.

**Figure 5.** Evaluation of the minimum inhibitory concentration (MIC) of fluconazole against the growth of *Candida albicans* at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 mg/ml.

**Table 2.** Minimum inhibitory concentration (MIC) of fluconazole against *Candida albicans* and *Candida glabrata*

| Row | Concentrations (mg/ml) | *Candida albicans* | *Candida glabrata* |
|-----|------------------------|--------------------|--------------------|
|     |                        | Fluconazole +/−    | Fluconazole +/−    |
| 1   | 0.5                    | -                  | -                  |
| 2   | 0.25                   | -                  | -                  |
| 3   | 0.12                   | -                  | -                  |
| 4   | 0.06                   | +                  | -                  |
| 5   | 0.03                   | +                  | -                  |
| 6   | 0.015                  | +                  | +                  |
| 7   | 0.007                  | +                  | +                  |
| 8   | 0.003                  | +                  | +                  |

* : growth
- : inhibited growth
Discussion

The present study aimed to investigate the antifungal effects of aqueous and alcoholic extracts of cumin on *Candida albicans* and *Candida glabrata*. The results of this study showed that alcoholic extract of cumin at a concentration of 100 mg/ml and aqueous extract of cumin at a concentration of 200 mg/ml inhibited the growth of *Candida albicans*. Alcoholic and aqueous cumin extracts inhibited the growth of *Candida glabrata* at a concentration of 200 mg/ml. Fluconazole inhibited the growth of *Candida albicans* and *Candida glabrata* at concentrations of 0.12 mg/ml and 0.03 mg/ml, respectively.

Therefore, both aqueous and alcoholic extracts of cumin inhibited the growth of *Candida albicans* and *Candida glabrata* in vitro. These findings are consistent with the results of studies by Gavanji et al (2015) [12], Haghigi et al (2011) [8], Naeini et al (2009) [13], Pai et al (2010) [14], and Ertürk (2006) [15]. Mohammadpour et al (2012) [16] investigated cumin essential oils. They showed that the composition of cumin essential oils can vary depending on many factors, including the geographic location of herb collection, the type of plantation, and harvest time [16]. Hajlaoui et al (2015) [17] also showed that other factors such as the used part of the plant, the type of the extraction method, and storage conditions make differences in the composition of cumin essential oils in addition to the geographic location of herb collection, the type of plantation, and harvest time. These factors also influence the extract samples. Cumin aldehyde, methane derivatives, c-terpinene, p-cymene, and b-pinene are the main components of several cumin essential oils and are mainly responsible for the aroma and the biological effects of cumin [17].

Both aqueous and alcoholic extracts of cumin were used in this study, which had antifungal effects on *Candida glabrata* and *Candida albicans*. Ertürk [15] showed the positive effect of cumin alcoholic extract on the inhibition of the growth of *Candida albicans*. Gavanji et al (2015) [12], Haghigi et al (2011) [8], Naeini et al (2009) [13], and Pai et al (2010) [14] showed the antifungal effect of cumin essential oil. Salari
et al (2012) [18] acknowledged that the antifungal effects of cumin on Candida species are due to high amounts of α-pinene, limonene, and cumin aldehyde (16.1%) in cumin essential oil. It seems that ethanolic extract is more efficacious than other extracts since alcohol renders a more pure extract with less polar compounds [19].

The favorable effects of a medicinal herb may be due to a combination of the main compounds of the plant rather than an active ingredient [20]. The difference in the MIC of alcoholic and aqueous extracts of cumin and essential oil of cumin can be due to different compounds of extracts and essential oils, the geographical location, environmental conditions of growth, difference in microbial growth, difference in the reaction of microorganisms to the essential oil, the solubility of the essential oil or its compounds, and use and the amount of the emulsifier [18].

Alcoholic extract of cumin at a concentration of 100 mg/ml and aqueous extract of cumin at a concentration of 200 mg/ml inhibited the growth of Candida albicans in this study. Alcoholic and aqueous extracts of cumin also inhibited the growth of Candida glabrata at a concentration of 200 mg/ml. Since the MIC of fluconazole was 0.12 mg/ml for inhibiting the growth of Candida albicans and 0.03 mg/ml for Candida glabrata in this study, it seems that the MIC of fluconazole is much smaller than those of aqueous and alcoholic extracts of cumin. This should be taken into account in the clinical use of cumin products.

Gavanji et al [12] compared the effect of essential oils with amphotericin B and ketoconazole on Candida albicans under laboratory conditions. Disk diffusion in agar and broth microdilution were used in the cited study. The results of the mentioned study showed that essential oils had the highest MIC (approximately 280 μg/ml) compared to other herbs and antifungal drugs [12]. Essential oil was used in the cited study, whereas ethanolic and aqueous extracts were used in the present study. Four plants were examined in the cited study. These four plants were collected from Mazandaran, Lorestan, and Chaharmahal and Bakhtiari provinces. In the present study, cumin samples were collected from Khorasan province. Therefore, the geographical location has a significant impact on the difference between the results of these two studies. Although the MICs of cumin antifungal drugs were greater than those of other herbs in the cited study, cumin showed antifungal effects in both studies.

Haghighi et al [8] evaluated the antifungal activity of essential oils of Thymus vulgaris, parsley cumin, and caraway against Candida albicans in comparison with fluconazole. Cumin was collected from a plant research center at 25 kilometers from north of Tehran. The MIC and the growth inhibition zone diameter were evaluated by the broth microdilution and disc diffusion methods, respectively. MIC 50, MIC 90, and the minimal lethal concentration were determined. The MIC 90 of cumin essential oils was determined to be 412 μg/ml [8]. Therefore, cumin essential oil showed antifungal effects on the standard strain of Candida albicans in the cited study, similar to the result of the present study. Differences in the results of the two studies may be due to the parts of the plant that were used in these studies. Essential oils were used in the mentioned study, while ethanolic and aqueous extracts were used in the present study. The location of plant collection also differed in the two studies. The samples were collected from a research center at northern Tehran in the mentioned study, while the samples were collected from Khorasan province in the present study.

Naeini et al [13] also studied the effects of essential oils and extracts of 50 Iranian herbs on Candida albicans standard strains under laboratory conditions. Seventeen essential oils and 172 extracts from 50 medicinal herbs used in traditional medicine in Iran were tested to investigate the antifungal and antibacterial effects of these herbs. Disk diffusion and agar dissemination were used in the mentioned study. The growth inhibition zone diameter of cumin essential oil was 45 mm, and this plant had very potent antifungal effects. No growth
inhibition zone was found when aqueous and ethanolic extracts of cumin were tested [13]. This may be due to the lack of dissemination of the extract in agar.

The antifungal effects of aqueous and ethanolic extracts of cumin on Candida albicans and Candida glabrata strains were studied using the microdilution method for the first time in our study. The interaction of agar with the microorganisms was eliminated in our study, and the extracts were directly in contact with the fungi; consequently, the inhibitory effect of the extracts on Candida albicans and Candida glabrata were directly visible. Ineffectiveness of aqueous and ethanolic extracts in the cited study can be due to the low concentrations of these extracts in the disks.

Pai et al [14] investigated the antifungal effects of four plants, including cumin, on Candida albicans under laboratory conditions. The agar disc diffusion method was used. The mean diameters of the growth inhibition zone after 24 and 48 hours were reported to be 1.81 mm and 6.5 mm, respectively. Cumin had less potent antifungal properties compared to other tested herbs [14]. The antifungal properties of cumin were also acknowledged in the present study. However, similar methods were not used to investigate the antifungal properties in these two studies. The plant part and the location of sample collection also differed in these two studies.

Naeini et al [13] studied the antifungal activity of some Iranian herbs used in traditional medicine against Candida strains. Disc diffusion in agar and broth microdilution were used in the cited study. The MIC was 280μg/ml, and the growth inhibition zone diameter was equal to 50 mm. Therefore, essential oils of some herbs, including cumin, have a very potent antifungal effect against Candida strains [13]. Cumin exhibited antifungal effects in the present study. Since the composition of this plant differs in various regions and similar concentrations were not investigated in the two studies, the MIC also differed. Essential oil was used in the cited study, while aqueous and alcoholic extracts were used in the present study.

Ertürk [15] investigated the antifungal and antibacterial activities of ethanolic extracts of 11 flavoring plants. Cumin samples were collected from a grocery store in Turkey. The disc diffusion and agar dilution methods were used in the mentioned study. The results showed that the MIC of cumin was 15 mg/ml, and the growth inhibition zone diameter was 14 mm [15]. The antifungal properties of cumin were also confirmed in the present study; nevertheless, different methods were used in these two studies. Ertürk [15] used the agar dilution method, while the microdilution method was used in the present study and agar involvement was eliminated. The location of plant collection was also different in these two studies.

Kamble (2015) [21] evaluated the antifungal activity of cumin against clinical isolates of Candida species. He used disc diffusion, broth microdilution, and broth macrodilution methods. The conclusion was that cumin can be a promising and effective natural therapeutic agent against Candidiasis [21].

Patil et al (2015) [22] studied the effect of cumin alone and in combination with routine antifungal drugs. Cumin showed effective results against Candida and synergism with conventional drugs; therefore, it can be used in combination with these drugs to reduce toxicity [22]. In an in-vitro study, Naeini et al (2014) [23] also showed the therapeutic effect of cumin on Candida species.

Conclusion

According to the findings of this study, both aqueous and alcoholic extracts of cumin exhibited measurable inhibitory activities against Candida species. The alcoholic extract was able to prevent the growth of Candida albicans at lower concentrations compared to the aqueous extract. The MICs of cumin aqueous and alcoholic extracts against Candida glabrata were equal. Alcohol and aqueous extracts of cumin inhibited the growth of Candida albicans and Candida glabrata at higher concentrations compared to fluconazole. One can hope that in the future, this herb can be used as a medicinal plant with minimal side effects.
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