

# *In vitro* Anti-Fungal Activity of *Cuminum cyminum* (Cumin Seed) Essential Oil against Clinical Isolates of *Candida* Species

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## ABSTRACT

**Objective:** The objective of the present investigation was to assess the *In vitro* inhibitory activity of essential oil extracted from *Cuminum cyminum* (cumin seed oil) against clinical isolates of *Candida albicans* and non-*albicans Candida*.

**Methods:** The cumin seed oil used in the present investigation was procured from Rakesh Sandal Products, Kanpur (U. P., India). The anticandidal activity of the essential oil was assessed against 75 clinical isolates of *Candida albicans* and non-*albicans Candida*. Sensitivity profile of clinical isolates to undiluted and diluted (3:1, 2:2 and 1:3) cumin seed oil was evaluated by disc diffusion method. Broth microdilution and broth macrodilution methods were used to evaluate the minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC).

**Results:** Cumin seed oil strongly inhibited all clinical isolates of *C. albicans* and non-*albicans Candida* with growth inhibition zones ranging from 27 to 72 mm. Cumin seed oil inhibited *C. albicans* growth with mean minimum inhibitory concentration (MIC) of 0.43  $\mu\text{l/ml}$  (v/v) and 0.32  $\mu\text{l/ml}$  (v/v) by broth micro dilution and broth macrodilution method, respectively. The clinical isolates of *C. albicans* required as high as 0.62  $\mu\text{l/ml}$  (v/v) concentration of cumin seed oil for its inhibition by both methods. The isolates of non-*albicans Candida* showed MIC range of 0.02 – 1.25  $\mu\text{l/ml}$  (v/v) by broth microdilution and broth macrodilution method. The majority of isolates of non-*albicans Candida* were inhibited at 0.62  $\mu\text{l/ml}$  (v/v) and 0.31  $\mu\text{l/ml}$  (v/v) by broth micro dilution and broth macrodilution method, respectively.

**Conclusion:** Cumin seed oil is an effective natural anticandidal agent that shows significant promise as a potential therapeutic agent for the treatment of superficial and mucosal candidiasis including vaginal candidiasis. Thus cumin seed oil can be effectively utilized for the control of *Candida* yeast.

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## INTRODUCTION

Multidrug resistance (MDR) is a serious and world-wide problem in the treatment of the opportunistic fungal infections that frequently badly affect immunosuppressed patients. Many antifungal drugs are available to fight deadly fungal infections, but many of these fungal pathogens have acquired multidrug resistance. In the developing countries, drugs are not only expensive but also have many side effects. Now in this period it is being emphasized to search medicinally valuable plants. Since earliest times medicinal plants have played a vital role in the development and comfort of human civilization. Many of the plants have medicinal properties that reduce symptoms or prevent diseases<sup>1</sup>. In present investigation interest has focused on *Cuminum cyminum* which is an ancient spice used in many countries. It contains medicinally important essential oil called cumin oil in seeds. Cumin (*Cuminum cyminum*) is one of the commonly used spices in food preparations. It is also used in traditional medicine as a stimulant, a stomachic, a carminative, and an astringent, and is useful in diarrhoea and dyspepsia. *Cuminum cyminum* seed oil is used instead of the seeds in many types of flavouring compounds, especially in curries and culinary preparations of oriental character. The cumin seed oil is used in perfumery and for imparting flavour to liquors and cordials<sup>2</sup>.

Essential oils are volatile distillates of plants containing several constituents responsible for biological activity of herbs and spices, and have been used medicinally in history especially for their antimicrobial nature. Several *In vitro* studies have established antimicrobial nature of essential

oils against bacteria, moulds and yeast<sup>3</sup>. Cumin seed oil is pale yellow in colour and cuminaldehyde (20% - 40%) is the chief constituent of the oil<sup>4</sup>. The other constituents of the oil are cuminol, carvone, cynol and terpenes<sup>5</sup>.

In the past two decades increased prevalence of fungal infections has been reported. Fungi cause both superficial and internal mycoses. Systemic fungal infections constitute a major community health problem in many parts of the world, both in developed and developing countries. *Candida* spp. is an important cause of blood infections, vaginal candidiasis and opportunistic infections in the oral cavity of immuno-compromised patients<sup>6</sup>. *Candida albicans* are responsible for almost all types of mucosal candidiasis and accountable for about 60% of superficial and systemic mycoses<sup>7</sup>. However, in current years this situation is further complexed by the appearance of non-albicans *Candida* (NAC) species such as *Candida glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* which cause serious oropharyngeal candidiasis and occasionally esophageal candidiasis<sup>8</sup>. Unfortunately, the increased use of antifungals in prophylactic and empirical therapy in high-risk patients has led to the development of drug-resistance in *Candida*.

Epidemiological studies also revealed rise in local fungal infections of chronic wounds (diabetic foot, burn, bed-sore, cancer ulceration), with the involvement of *C. albicans* biofilm. Such infections are very difficult to eradicate since *Candida* cells living as a biofilm community show extremely high resistance to most currently used antifungal drugs<sup>9</sup>.

The increasing ineffectiveness of these drugs and unavailability of alternative antimicrobials in developing countries causing spread of major infectious diseases. This necessitated the search of new antimicrobial substances from medicinal plants. The objective of the present study was to assess the *In vitro* inhibitory potential of cumin seed oil against clinical isolates of *Candida albicans* and non-*albicans Candida*.

## MATERIALS AND METHODS

### Essential oil

The cumin seed oil used in the present studies was procured from Rakesh Sandal Products, Kanpur (U. P., India) in sealed glass bottle. Essential oil was subjected to sterility checking by inoculating a loopful of oil on potato dextrose agar and nutrient agar slants, and then assessing the growth. The essential oil was stored in the dark at 25°C when not in use. Different concentrations of cumin seed oil with DMSO (25%, 50% and 75%) were prepared for experiments.

### Organisms

75 clinical isolates of the pathogenic yeasts including *Candida albicans* (n=28), *C. krusei* (n=7), *C. glabrata* (n=9), *C. parapsilosis* (n=7), *C. tropicalis* (n=12), *C. pseudotropicalis* (n=6), *C. guilliermondii* (n=3) and *C. stellatoidea* (n=3) were isolated from various clinical specimens such as oral swab, vaginal discharge and blood. Each isolate was originated from a different patient with clinical manifestations. Clinical isolates were identified to the species level based on morphological criteria, carbohydrate assimilation profile, and germ tube test in serum and chlamydospores production on corn meal agar<sup>10</sup>. Isolates were maintained on Sabouraud dextrose agar (Hi-Media, Mumbai) at 4°C in refrigerator until used in

the study. Prior to testing, each isolate was checked for purity and viability.

### Inoculum preparation

To prepare inoculum<sup>11</sup>, a small amount of growth was taken from 24 h old culture of the respective clinical isolate grown on SDA slant and inoculated into 5 ml of 0.85% sterile saline. The resulting inoculum density was adjusted to 0.5 McFarland standard to obtain a suspension of  $1 \times 10^6$  to  $5 \times 10^6$  cells/ml.

### Anticandidal sensitivity testing

Disc diffusion method as described by Bauer *et al.* (1966)<sup>12</sup> was used for anticandidal sensitivity testing. The plates (90 mm diameter) containing Sabouraud dextrose agar (Hi-Media, Mumbai) at a depth of 4 mm were used. The agar surface was streaked with inoculum by using a sterile swab. Sterile disc (SD 067, Hi-Media, Mumbai) of 6 mm diameter was impregnated with 20 µl of undiluted (100%) and diluted (25%, 50%, 75% in DMSO) oil was placed at center of seeded agar surface. The plates were then kept undisturbed for 30 min to allow diffusion of the essential oil into the agar and incubated at 35 °C for 24 h. The inhibition zone was measured in millimeter with zone reader scale and the assay was carried out in triplicate for each isolate tested. Isolates with zone size  $\geq 28$  mm were classified as strongly sensitive, with a zone diameter of  $< 28$  to 16 mm as moderately sensitive, with a zone diameter of  $< 16$  to 12 mm as weakly sensitive and isolates with zone diameter of  $< 12$  mm as resistant<sup>13</sup>.

### MIC & MFC studies

Minimum inhibitory concentrations (MIC) and Minimum fungicidal concentrations (MFC) of cumin seed oil were determined by broth microdilution and

broth macrodilution method<sup>11</sup> with some modifications.

#### Broth microdilution method

Stock solution of cumin seed oil (10 µl/ml) was prepared in sterile Sabouraud dextrose broth (Hi-Media, Mumbai). 0.15% (w/v) bacteriological agar was added as a stabilizer<sup>14</sup> of the oil water mixture. Serial two fold dilutions of stock solution of essential oil was prepared over the range of 0.02–10 µl/ml (v/v) with a final oil concentration range 0.01–5.0 µl/ml (v/v) in 96-well microtitre plates. A freshly grown yeast suspension in Sabouraud dextrose broth was standardized to  $1 \times 10^6$  cells/ml (0.5 McFarland standard). A working yeast inoculum suspension of  $1 \times 10^4$  cells/ml was prepared by diluting the stock inoculum ( $1 \times 10^6$  cells/ml) 1:100 with sterile Sabouraud dextrose broth. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. 100 µl yeast suspension was added to each well. Well containing only the Sabouraud dextrose broth with 0.15% agar without microorganism was used as sterility control. The lowest concentration of oil that inhibited visible growth after incubation at 35°C for  $48 \pm 2$  h without shaking was taken as minimum inhibitory concentration (MIC). Minimum concentration of oil that inhibited 50%, 70% and 90% of the isolates tested were defined as MIC<sub>50</sub>, MIC<sub>70</sub> and MIC<sub>90</sub>, respectively. To determine minimum fungicidal concentration (MFC), a loopful of broth was removed from each well and spot inoculated onto SDA plate and after incubation at 35°C for 48 h, the lowest concentration of oil that inhibited the complete growth was considered as MFC. The concentration of cumin seed oil fungicidal for 90% of the clinical isolates tested was defined as MFC<sub>90</sub>.

#### Broth macrodilution method

A range of doubling dilutions of cumin seed oil from 0.02 – 10 µl/ml (v/v) with a final oil concentration range 0.01 – 5 µl/ml (v/v) was prepared in Sabouraud dextrose broth in round bottom sterile glass tubes (12 × 75 mm). Bacteriological agar was added at a concentration of 0.15% (w/v) to enhance oil solubility. A working inoculum suspension of  $1 \times 10^4$  cells/ml was added to each tube except sterility control. Sabouraud dextrose broth containing 0.15% agar without essential oil served as growth control. The tubes were then incubated at 35°C for  $48 \pm 2$  h without agitation and observed for the presence or absence of visible growth. The MIC was defined as the lowest concentration of oil inhibiting visible growth.

## RESULTS

#### Anticandidal activity by disc diffusion

In the present investigations, anticandidal activity of cumin seed oil was evaluated *In vitro* by disc diffusion method. The mean inhibition zones (MIZ) and inhibition zone range (IZR) obtained against eight different *Candida* species is shown in Table 1. Undiluted cumin seed oil strongly inhibited all the clinical isolates of *Candida* with 28 – 72 mm inhibition zone range with average inhibition zone of 52 mm. Oil of cumin seed was highly potent against the isolates of *Candida albicans* (MIZ=57) and was comparatively least potent to the isolates of *C. parapsilosis* (MIZ = 38 mm). Isolates of *C. tropicalis* and *C. pseudotropicalis* were inhibited with mean inhibition zone of 54 mm, while the isolates of *C. krusei* and *C. guilliermondii* were inhibited with mean inhibition zone of 53 mm and 52 mm, respectively. All the clinical isolates were sensitive even to the 3:1, 2:2 and 1:3 diluted cumin seed oil.

### Evaluation of anticandidal activity by broth microdilution method

Different concentrations of cumin seed oil have been used to determine the MIC<sub>90</sub> and MFC<sub>90</sub> by broth microdilution method (Table 2). The MIC range from 0.08 µl/ml to 1.25 µl/ml was found to be highly effective for inhibiting all *Candida* species. *C. krusei* and *C. stellatoidea* was the most susceptible among the *Candida* species, requiring lowest amount of cumin seed oil for its inhibition, with its MIC<sub>90</sub> at 0.31 µl/ml. The least susceptible *Candida* species were the *C. glabrata*, *C. parapsilosis*, *C. pseudotropicalis* and *C. guilliermondii*, with MIC<sub>90</sub> of 1.25 µl/ml. MIC range for the isolates of *C. albicans* was found to be 0.08–1.25 µl/ml cumin seed oil with MIC<sub>90s</sub> at 0.62 µl/ml. Much of the isolates of *C. tropicalis* showed good consistency with respect to MIC requirement, with exception of few isolates that showed 2 fold variations in their MIC values. MIC<sub>90</sub> of *C. albicans*, *C. stellatoidea*, *C. pseudotropicalis*, *C. parapsilosis* and *C. glabrata* were two fold greater than MIC<sub>50</sub>, while MIC<sub>90</sub> of *C. tropicalis*, *C. krusei* and *C. guilliermondii* was found to be equal to MIC<sub>50</sub>.

### Evaluation of anticandidal activity by broth macrodilution method

Table 3 illustrated the MIC and MFC of cumin seed oil assessed by broth macrodilution method at different concentrations. The broth macrodilution studies also exhibited higher susceptibility of *C. krusei* and *C. stellatoidea* to cumin seed oil with MIC<sub>90</sub> of 0.31 µl/ml. The broth macrodilution method demonstrated the uniform susceptibility of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. pseudotropicalis*, all requiring 0.62 µl/ml concentration of cumin seed oil for inhibiting 90% of the clinical isolates. *C. guilliermondii* was found to be the least susceptible species requiring 1.25 µl/ml

concentration of cumin seed oil as their MIC<sub>90</sub>. The wide range of MIC (0.02 – 1.25 µl/ml) was noted for the isolates of *C. glabrata*. MIC<sub>90</sub> of *C. albicans*, *C. glabrata* and *C. tropicalis* were found to be two fold greater than MIC<sub>50</sub>, while MIC<sub>90</sub> of *C. parapsilosis*, *C. pseudotropicalis*, *C. krusei*, *C. guilliermondii* and *C. stellatoidea* was found to be similar to MIC<sub>50</sub>.

## DISCUSSION

*C. albicans* causes superficial as well as life threatening systemic infections under immunocompromised states<sup>10</sup>. *Candida* is an opportunistic microorganism and cause issues like thrush, vaginal yeast infections, and systemic candidiasis. In this context, aim was to evaluate the possible therapeutic potential of cumin seed oil against this opportunistic human pathogen, which can become a facultative pathogen under altered physiological situations. The emergence of antifungal resistance among *Candida albicans* and related species is a major problem for treatment of candidiasis. In the light of this information and with an intension to manage the *C. albicans*, our previous investigation<sup>15</sup> assessed the antifungal activity of several essential oils and reported the strong antifungal activity of cumin seed oil against reference strains of *C. albicans* (MTCC-227, MTCC-3017 & NCIM- 3100), *C. glabrata* MTCC 3019, *C. krusei* MTCC 231, *C. blanki* MTCC 624, *C. cylindracea* MTCC 1908 and *C. tropicalis* MTCC 184, with inhibition zones ranging from 30 to 60 mm. Previous studies by Naeini *et al.*<sup>16</sup>, also demonstrated the *In vitro* antifungal activities of essential oil from *Cuminum cyminum* (cumin seed oil) against reference strains of *C. albicans* ATCC 14053, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 and reported a broad-spectrum antifungal activity of *C. cyminum* oil against different pathogenic *Candida* species, with

inhibition zone values ranging from 7 to 50 mm. In the present investigations therapeutic potential of cumin seed oil is assessed directly against the *Candida* spp isolated from different patient with clinical manifestations. The results were extremely promising and demonstrated the potential of *C. cyminum* essential oil as natural inhibitors to control the most important pathogenic *Candida* species and substitute therapies for candidiasis in place of present antifungal chemicals.

The increasing incidences of systemic fungal infections are causing consequent increase in mortality. The management of *Candida* infections is difficult due to increased resistance of *Candida* isolates to commercial antifungal drugs. Relapse of *Candida* infections, high cost and limited number of effective antifungal drugs, toxicity and side effects of the available antifungal drugs are the major factors contributing towards increase of antifungal resistance. Thus, there is a urgent need to search for new compounds from other sources, including medicinal plants to act against these microorganisms with respect to spectrum, potency, safety and pharmacokinetic properties, but with a selective and little or no toxic effect<sup>17,18</sup>. In the present investigations, cumin seed oil showed effective inhibition of all clinical isolates of *Candida* species at a concentration as low as 0.02 µl/ml, with a maximum inhibitory concentration 1.25 µl/ml. The low fungicidal concentration of cumin seed oil against pathogenic yeast may provide an exciting potential in future, especially in the light of the going away from synthetic drugs therapy and the move towards more natural alternative. In majority of *Candida* isolates, MIC values by broth microdilution and broth macrodilution method were either same or differed by two-fold dilution. The agreement between broth microdilution and broth macrodilution

method was good in regards to the reproducibility of results.

Cumin seed oil have strong potential to control the production of Aflatoxins which are highly toxic and carcinogenic metabolites produced by fungus *Aspergillus parasiticus* on food and agricultural products. Khosravi *et al.*,<sup>19</sup> studied the effect of *Cuminum cyminum* essential oil on growth and aflatoxins production by *A. parasiticus* and reported the strongest inhibition of *A. parasiticus* growth and its aflatoxin production. Thus essential oil of *C. cyminum* could be used at low concentrations to protect foods from fungal and toxin contaminations by *A. parasiticus*. Cumin seed oil also have a potential as a plant based shelf life enhancer against fungal and aflatoxin contamination and possess efficacy as a preservative in stored foods<sup>20</sup>. In view of the antifungal and antiaflatoxigenic nature, free radical scavenging potential and efficacy in food system, cumin seed oil may be able to provide protection of food commodities against quantitative and qualitative losses<sup>20</sup>.

Several researchers carried out chemical analysis of cumin seed oil by GC-MS. Rafeef<sup>21</sup> reported 1H-indene derivatives (59.77%) and Cumenic aldehyde (13.77%) as a major compound in the extracted oil. Wanner *et al.*<sup>22</sup> analysed the cumin oil samples from four different geographical origins and reported the beta-pinene, p-cymene, gamma-terpinene, terpenoid aldehydes, cumenic aldehyde and the menthadien carboxaldehydes as the major compounds in cumin oils. The major components reported by Lu Wang *et al.*<sup>23</sup> included cuminal, cumin-alcohol, p-cymene, β-pinene, 2-care-10-al, and γ-terpinene. Ridawati *et al.*<sup>24</sup> reported the dominance of 4 compound i.e. cuminaldehyde (35.44%), p-cymene (34.77%), β-pynene(15.08 %) and γ-terpinene (8.15%) in cumin seed oil. Cuminaldehyde, a main constituent of cumin

oil is an important phytochemical and possesses many health benefits. Other compounds reported from cumin seed oil are limonene, 1, 8-cineole, linalool, linalyl acetate, and  $\alpha$ -terpineole<sup>25</sup>. Shetty *et al.*,<sup>26</sup> reported more sensitivity of fungal cultures (*Aspergillus*, *Penicillium* spp, *Saccharomyces* and *Candida* spp.) to cumin oil and cuminadehyde than bacteria. The observed toxicity of cumin oil in present investigations against different species of *Candida* may be due to the presence of cuminaldehyde in cumin seed oil. Present study indicates that *C. cyminum* L. has considerable anti-*Candida* activity and thus deserves further investigation for clinical applications.

Clinical Laboratory Standard Institute (CLSI)<sup>11</sup> have published approved protocol for antifungal susceptibility testing by broth microdilution and disc diffusion assay. Diffusion methods are only useful as a qualitative screening method<sup>27</sup>. The diameter of zone of inhibition depends on the ability of the test substance to diffuse uniformly through an agar medium. Most essential oils and their active compounds are highly volatile and show poor solubility in the aqueous phase<sup>28</sup>. In present studies, 0.15% bacteriological agar as suggested by Mann and Markham<sup>14</sup> was used to emulsify cumin seed oil into the test medium and results showed good solubility of oil into the test medium after the addition of 0.15% bacteriological agar. Dilution methods give more quantitative results. MICs depend on many factors such as temperature and time of incubation and the size of test inoculum. However, there is a need to standardize susceptibility test methods for essential oils to increase reproducibility.

Oropharyngeal Candidiasis (OPC) is a most common opportunistic fungal disease in HIV/AIDS patients globally. As antimicrobial agents, essential oils may be appropriate in HIV/AIDS for specific

opportunistic infections. Essential oil could be explored for potential use in HIV/AIDS focusing on opportunistic infections caused by *Candida albicans* and others<sup>29</sup>. Although there is no known cure for HIV, the immune system can be strengthened and secondary infection can be prevented through the use of essential oils. There is limited information available about the use of essential oils in the care of AIDS. Salari *et al.*<sup>30</sup> evaluated effect of *Cuminum cyminum* oil on the *Candida albicans* isolated from HIV patients in Iran and reported to have strong inhibitory activity on Fluconazole-susceptible and Fluconazole-resistant *Candida albicans* isolates. Thus the antifungal effect this essential oil against *Candida* species could be explored for the control of fungal diseases in immunocompromised AIDS patients. In present investigation cumin seed oil strongly inhibited all the isolates of *Candida* species isolated from various clinical specimens such as oral thrush, vaginal discharge and blood, with MICs as high as 1.25  $\mu$ l/ml. For *C. albicans* MIC range was found to be 0.08–1.25  $\mu$ l/ml by broth microdilution method, while the range was 0.08–0.62  $\mu$ l/ml by broth macrodilution method. Evidences suggest that cumin seed oil have antifungal activity against *Candida*. However, well-designed trials are needed before a firm conclusion can be made.

Antibacterial activity of essential oil of *Cuminum cyminum* is also acknowledged in literature. Results of Syed *et al.*<sup>31</sup> showed marked activity of cumin seed oil against the bacterial pathogens at quite low concentration (800-1200 ppm). Mominul<sup>32</sup> reported antibacterial activity of cumin seed extracts against gram positive and gram negative human pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. *C. cyminum* essential oil is also effective for the control of *Lactococcus garvieae* a fish

pathogen that causes lesions in the vascular endothelium, leading to hemorrhages and petechias at the surface of internal organs<sup>33</sup>. Cumin (*Cuminum cyminum*) Seed essential oil is reported to have antiseptic activity. Motamedifar Mohammad<sup>34</sup> reported significant antiviral activity of methanolic extract of cumin seed on herpes simplex virus (HSV-1) in Vero cell line. The essential oil of *Cuminum cyminum* was also found to be most effective for antiviral activity against papaya ring spot poty virus<sup>35</sup>. Cumin seeds and its essential oil could be used as a source of new antibacterial agent for developing drugs to inhibit some human pathogens. The potent anticarcinogenic effect of cumin essential oil has also been reported earlier<sup>36</sup>.

Shrama and Singh<sup>37</sup> and Saxena<sup>38</sup> reported the toxicity of essential oil of *Cuminum cyminum* (cumin) to *Aspergillus candidus*, *A. flavus*, *A. nidulans*, *A. niger*, *Cladosporium herbarum*, *Fusarium* sp., *Helminthosporium sacchari*, *Microsporium* sp., *Mucor mucedo*, *Rhizopus* sp., *Alternaria* sp., *Trichophyton* sp. *Candida albicans*, *C. tropicalis* and *Keratinomyces ajelloe*. Shetty *et al.*,<sup>26</sup> reported more sensitivity of fungal (*Aspergillus* and *Penicillium* sp.) and yeast (*Saccharomyces* and *Candida* sp.) cultures to cumin oil and cuminaldehyde than bacteria. Present study showed strong inhibitory action of cumin oil against all species of *Candida* tested. Thus agreement with these earlier reports of Sharma and Singh<sup>37</sup>, Saxena<sup>38</sup> and Shetty *et al.*,<sup>26</sup> was seen regarding toxicity of cumin oil to *C. albicans*, and *C. tropicalis*.

## CONCLUSION

In conclusion, cumin oil is an effective natural anticandidal agent that shows significant promise as a potential therapeutic agent for the treatment of superficial and mucosal candidiasis including vaginal candidiasis. Thus, cumin

seed oil can be helpful as natural inhibitors to control the growth of the most important pathogenic *Candida* species and alternative therapies for candidiasis. *In vitro* results indicate anticandidal efficacy of cumin oil at low concentration. But certain clinical trials are needed to determine the efficacy of cumin oil *in vivo*. The results showed the potential of cumin seed oil as cheap and convenient substitution to pharmaceutical antifungal products.

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**Table 1.** Inhibition zones obtained by disc diffusion method of the cumin seed oil assayed against eight different *Candida* species

Species (No. of isolates)	Inhibition Zones (in mm)							
	4:0 (100% oil)		3:1 (75% oil)		2:2 (50% oil)		1:3 (25% oil)	
	MIZ	IZR	MIZ	IZR	MIZ	IZR	MIZ	IZR
<i>Candida albicans</i> (n = 28)	57	44 – 72	52	40 – 67	47	37 – 60	41	33 – 52
<i>C. glabrata</i> (n = 9)	46	30 – 68	42	27 – 63	39	23 – 60	35	21 – 55
<i>C. tropicalis</i> (n = 12)	54	35 – 72	50	45 – 67	46	32 – 62	41	28 – 53
<i>C. parapsilosis</i> (n = 7)	38	28 – 44	33	22 – 40	29	20 – 38	26	19 – 34
<i>C. pseudotropicalis</i> (n = 6)	54	45 – 64	49	43 – 55	46	39 – 51	46	36 – 47
<i>C. krusei</i> (n = 7)	53	50 – 56	50	44 – 54	48	41 – 52	42	39 – 44
<i>C. guilliermondii</i> (n = 3)	52	44 – 60	47	39 – 55	43	35 – 51	39	32 – 46
<i>C. stellatoidea</i> (n = 3)	49	48 – 50	43	42 – 45	40	39 – 40	36	35 – 36
Total (n = 75)	52	28 – 72	48	22 – 67	43	20 - 62	39	19 – 55

MIZ, Mean Inhibition Zone; IZR, Inhibition Zone Range; 4:0 represents undiluted oil; 3:1 represents 3 parts oil and 1 part DMSO solvent; 2:2 represents 2 parts oil and 2 parts DMSO solvent; 1:3 represents 1 part oil and 3 parts DMSO solvent. Data are means of triplicates determinations.

**Table 2.** Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) obtained by broth microdilution

Species (No. of isolates)	MIC ( $\mu\text{l/ml}$ )					MFC ( $\mu\text{l/ml}$ )
	MIC Range	MIC <sub>50</sub>	MIC <sub>70</sub>	MIC <sub>90</sub>	Mean MIC	MFC <sub>90</sub>
<i>Candida albicans</i> (n = 28)	0.08 – 1.25	0.31	0.62	0.62	0.43	0.62
<i>C. glabrata</i> (n = 9)	0.15 – 1.25	0.62	1.25	1.25	0.85	1.25
<i>C. tropicalis</i> (n = 12)	0.31 – 0.62	0.62	0.62	0.62	0.50	0.62
<i>C. parapsilosis</i> (n = 7)	0.31 – 1.25	0.62	1.25	1.25	0.74	1.25
<i>C. pseudotropicalis</i> (n = 6)	0.31 – 1.25	0.62	0.62	1.25	0.70	1.25
<i>C. krusei</i> (n = 7)	0.15 – 0.31	0.31	0.31	0.31	0.25	0.31
<i>C. guilliermondii</i> (n = 3)	1.25 – 1.25	1.25	1.25	1.25	1.25	1.25
<i>C. stellatoidea</i> (n = 3)	0.15 – 0.31	0.15	0.15	0.31	0.23	0.31
Total (n = 75)	0.08 – 1.25	1.25	1.25	1.25	0.61	1.25

MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration; (MIC and MFC values are expressed in  $\mu\text{l/ml}$ ). Data are means of triplicates determinations.

**Table 3.** Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) obtained by broth macrodilution

Species (No. of isolates)	MIC ( $\mu\text{l/ml}$ )					MFC ( $\mu\text{l/ml}$ )
	MIC Range	MIC <sub>50</sub>	MIC <sub>70</sub>	MIC <sub>90</sub>	Mean MIC	MFC <sub>90</sub>
<i>Candida albicans</i> (n = 28)	0.08 – 0.62	0.31	0.31	0.62	0.32	0.62
<i>C. glabrata</i> (n = 9)	0.02 – 1.25	0.31	0.62	0.62	0.52	0.62
<i>C. tropicalis</i> (n = 12)	0.08 – 0.62	0.31	0.31	0.62	0.33	0.62
<i>C. parapsilosis</i> (n = 7)	0.31 – 0.62	0.62	0.62	0.62	0.49	0.62
<i>C. pseudotropicalis</i> (n = 6)	0.31 – 0.62	0.62	0.62	0.62	0.54	0.62
<i>C. krusei</i> (n = 7)	0.08 – 0.31	0.31	0.31	0.31	0.23	0.31
<i>C. guilliermondii</i> (n = 3)	1.25 – 1.25	1.25	1.25	1.25	1.25	1.25
<i>C. stellatoidea</i> (n = 3)	0.31 – 0.31	0.31	0.31	0.31	0.31	0.31
Total (n = 75)	0.02 – 1.25	1.25	1.25	1.25	0.49	1.25

MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration; (MIC and MFC values are expressed in  $\mu\text{l/ml}$ ). Data are means of triplicates determinations.