# Antifungal Effect of *Cuminum cyminum* Extract on Candida albicans ATCC 10231 (Experimental Laboratory)

Anis Dien Hartini[1], Primodanti Jiwanakusuma[1], Anggraini Margono[2]*, Endang Suprastiwi[2]

1. Conservative Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
2. Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

**Abstract**

*Candida albicans* causes persistent endodontic infections; however, current antifungal materials for *C. albicans* infection have some limitations. Because *Cuminum cyminum* (cumin) has antifungal properties, its efficacy against *C. albicans* ATCC 10231 was investigated using Sabouraud Dextrose Broth (SDB).

Steam distillation technique was used to obtain 5 different concentrations of cumin extract (0.1, 0.2, 0.4, 0.8, and 1.6 µL/mL). To evaluate the antifungal effect of cumin extract, the turbidity of *C. albicans* was assessed with an enzyme-linked immunosorbent assay (ELISA) reader. The cumin extract concentration that resulted in the lowest turbidity value of *C. albicans* ATCC 10231 was 0.4 µL/mL, which was significantly different from those produced by concentrations 0.1, 0.2 µL/mL, and the control.

The concentration that resulted in the lowest turbidity of the biofilm was 0.1 µL/mL; however, this finding was not significantly different from biofilm turbidity values of other groups. Turbidity was lower for the planktonic form than for the biofilm which was significantly different from those produced by concentrations 0.4, 0.8, and 1.6 µL/mL.

In conclusion cumin extract has an antifungal effect on both planktonic and biofilm forms of *C. albicans* ATCC 10231. Furthermore, it is more effective against planktonic than biofilm form.

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## Introduction

Fungi are associated with endodontic infections.[1] *Candida albicans* is the most commonly persistent pathogen and has a role in the failure of endodontic treatment. The complex biofilm structure presence of a biofilm matrix, expression of drug efflux pumps, and presence of metabolic plasticity can increase *C. albicans* resistance to antifungal agents.[2] In addition, the synergistic interaction of *C. albicans* with *Streptococcus* and *Peptostreptococcus* micros plays a role in persistent endodontic lesions.

In endodontic treatment, commonly used antimicrobial agents are synthetic materials, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), and ethylenediaminetetraacetic (EDTA). The effectiveness of these materials is influenced by their concentration; the higher the concentration, the better the effectiveness.[3] However, these synthetic materials have several side effects such as allergic and toxic reactions.[4] NaOCl is known to cause some adverse reactions, such as chemical burns and necrosis of the periapex tissue; CHX is known to cause allergic reactions and discoloration of the teeth and gums. Furthermore, achieving effectiveness requires the use of a high concentration; however the higher the concentration, the greater the possibility of allergic and toxic reactions. Therefore, an effective and safe antifungal agent is warranted.

*Cuminum cyminum* is one of the herbs which is claimed to effectively inhibit the growth of both fungi and bacteria.5 The main element of cumin extract, *cuminaldehyde*, and minor elements *terpene* elements are known to have antimicrobial effects.[6] Naeini et al.[7] (2013) compared the effects of cumin extract antifungals with nystatin and siwak (*Salvadora persica*) and

***Corresponding author:***
**Anggraini Margono**
Department of Conservative Dentistry,
Faculty of Dentistry, Universitas Indonesia,
Jakarta, Indonesia.
E-mail: margonodewi@yahoo.com

showed that cumin extract had the best antifungal effect among the 3. Cumin extract can eliminate broad-spectrum fungi and some types of fungi that are resistant to synthetic antifungal agents.[7,8] We analyzed the effect of cumin extract against *C. albicans* ATCC 10231 in both planktonic and biofilm forms.

## Materials and methods

This study was approved by the Ethics Committee of Universitas Indonesia, Faculty of Dentistry (Ethics No.109/Ethical Approval/FKGUI/XI/2017-Protocol 051191017).

The laboratory experimental study consisted of several stages: preparation of cumin extract, isolation of *C. albicans*, antimicrobial test, and statistical analysis. There were 2 groups of *C. albicans*: the planktonic and biofilm groups. In addition to control group, the study included 5 test groups concentration of cumin extract: 0.1, 0.2, 0.4, 0.8, and 1.6 µL/mL. Each concentration was tested 5 times with the 2 *C. albicans* groups.

### Preparation of Cumin Extract

Cumin extract was prepared using the steam distillation technique and then stored in a dark room in a black bottle at 4ºC. We used gas chromatography-mass spectrometry (GC-MS) to analyze the active content of cumin extract.

### Isolation of C. albicans ATCC 10231

*C. albicans* ATCC 10231 was cultured in CHROMagar and incubated at 37ºC for 24 h. Next, it was inoculated into Sabouraud dextrose broth. The inoculum density was calculated using a hemocytometer to obtain a 1 x 106 cells/mL suspension.

### Crystal Violet Assay

To test the effectiveness of cumin extract on planktonic *C. albicans* 100 µL of a 1 x 106 cells/mL *C. albicans* inoculum was added into each well. Then, the test materials of different concentrations were added into the wells and subsequently incubated at 37ºC for 15 min. The amount of fungal growth was determined from the optical density (OD) spectrophotometrically read on the enzyme-linked immunosorbent assay (ELISA) reader.

To test the effectiveness of cumin extract on *C. albicans* biofilm 100 µL of 1 x 106 cells/mL suspensions of *C. albicans* was added into each well and then incubated at 37°C for 48 h to to allow biofilm formation on the well surface. Each well-plate was washed with phosphate buffer saline (PBS). Then, 200 µL of 0.1% crystal violet solution was added to each well and incubated for 15 min. Next, the crystal violet solution of each well-plate was discarded, and the well-plates were washed with PBS. Then, 100% ethanol (200 µL) was added to each well-plate, and OD was measured with an ELISA reader with a wavelength of 450 nm and 10s shaker time.

### Statistical Analysis

We used the SPSS software to analyze the data with the non-parametric statistical Kruskal-Wallis test ($P < 0.05$).

## Results

The analysis of cumin extract content with GC-MS showed that *cuminaldehyde* was the major component (61.65%). Other elements found in the cumin extract listed in Table 1.

The effectiveness test of cumin extract with crystal violet assay (Table 2) showed that OD was lowest for the concentration of 0.4 µL/mL and highest for 0.2 µL/mL. The test of cumin extract on the biofilm form of *C. albicans* revealed that the mean value of OD was lowest for the concentration of 0.1 µL/mL and highest for 0.8 µL/mL.

Table 3 shows that the cumin extract concentration of 0.4 µL/mL yielded significantly different results compared with the concentrations of 0.1 and 0.2 µL/mL and the control, indicating, that cumin extract at minimum concentration of 0.4 µL/mL can inhibit the planktonic growth of *C. albicans* ATCC 10231. In the biofilm test group, there was no significant difference ($P > 0.05$) among the results.

The antifungal effects of cumin extract on planktonic *C.albicans* were statistically compared with those on *C. albicans* biofilm. This analysis revealed significant differences in the effect of different concentrations of cumin extract, 0.4, 0.8, 1.6 µL/mL for planktonic and biofilm *C. albicans*. It also revealed that the antifungal effect of cumin extract was better against planktonic *C.albicans* than against biofilm *C. albicans*.

| Compounds | Percentage (%) |
|---|---|
| *Cuminaldehyde* | 61.65 |
| *Cumene* | 10.79 |
| *P-cymene* | 6.61 |
| *β-pinene* | 2.64 |
| *Acetic acid* | 1.93 |
| *P-cymen-7-ol* | 1.74 |
| *Pentanoic acid* | 1.66 |
| *Formic acid* | 1.23 |
| *γ-terpinene* | 1.12 |
| Others | 10.63 |

**Table 1.** Gas chromatography-mass spectrometry (GC-MS) analysis of the components of cumin extract.

| Research Group* | Cumin Extract Concentration (µL/mL) | N | Optival Density | | |
|---|---|---|---|---|---|
| | | | Average± SD | Lower Limit | Upper Limit |
| P1 | 0.1 | 6 | 0.02783 ± 0.001014 | 0.02523 | 0.03044 |
| P2 | 0.2 | 6 | 0.03817 ± 0.001558 | 0.03416 | 0.04217 |
| **P3** | **0.4** | **6** | **0.02083 ± 0.001376** | **0.01730** | **0.02437** |
| P4 | 0.8 | 6 | 0.02333 ± 0.000333 | 0.02248 | 0.02419 |
| P5 | 1.6 | 6 | 0.02917 ± 0.011429 | -0.00021 | 0.05855 |
| **P6** | † | **6** | **0.04183 ± 0.000946** | **0.03940** | **0.04426** |
| **B1** | 0.1 | **6** | **0.04417 ± 0.022300** | **-0.01316** | **0.10149** |
| B2 | 0.2 | 6 | 0.05267 ± 0.015669 | 0.01239 | 0.09294 |
| B3 | **0.4** | 6 | 0.10317 ± 0.025063 | 0.03874 | 0.16759 |
| **B4** | 0.8 | **6** | **0.12250 ± 0.034656** | **0.03341** | **0.21159** |
| B5 | 1.6 | 6 | 0.08100 ± 0.018217 | 0.03417 | 0.12783 |
| B6 | † | 6 | 0.10600 ± 0.017441 | 0.06117 | 0.15083 |

Kruskal-Wallis test of significance between the groups,with P <0.05, SD: standard deviation.
*B: C. albicans biofilm; P: C. albicans planktonic form.
† negative control

**Table 2.** Average optical density value of whole cumin extract concentration with planktonic and biofilm Candida albicans ATCC 10231.

| Research Group | Cumin Extract Concentration (µL/mL) | Research Group | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P1 | P2 | P3 | P4 | P5 | P6 | B1 | B2 | B3 | B4 | B5 | B6 |
| P1 | 0.1 | | **0.004*** | **0.01*** | **0.007*** | 0.054 | **0.004*** | 1 | | | | | |
| P2 | 0.2 | **0.004*** | | **0.004*** | **0.004*** | 0.053 | 0.108 | | 0.335 | | | | |
| P3 | 0.4 | **0.01*** | **0.004*** | | 0.052 | 0.376 | **0.004*** | | | **0.006*** | | | |
| P4 | 0.8 | **0.007*** | **0.004*** | 0.052 | | 0.104 | **0.004*** | | | | **0.007*** | | |
| P5 | 1.6 | 0.054 | 0.053 | 0.376 | 0.104 | | 0.054 | | | | | **0.025*** | |
| P6 | † | **0.004*** | 0.108 | **0.004*** | **0.004*** | 0.054 | | | | | | | |
| B1 | 0.1 | 1.000 | | | | | | | 1.000 | 1.000 | 0.346 | 1.000 | 1.000 |
| B2 | 0.2 | | 0.335 | | | | | 1.000 | | 1.000 | 0.615 | 1.000 | 1.000 |
| B3 | 0.4 | | | **0.006*** | | | | 1.000 | 1.000 | | 1.000 | 1.000 | 1.000 |
| B4 | 0.8 | | | | **0.007*** | | | 0.346 | 0.615 | 1.000 | | 1.000 | 1.000 |
| B5 | 1.6 | | | | | **0.025*** | | 1.000 | 1.000 | 1.000 | 1.000 | | 1.000 |
| B6 | † | | | | | | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | |

* Kruskal-Wallis test of significance between the groups, with P <0.05.
* B: C. albicans biofilm; P: C. albicans planktonic form.
†negative control.

**Table 3.** Significance of optical density value of cumin extract on planktonic and biofilm Candida albicans ATCC 10231.extract is generally due to the main components, but the minor components present in the extract may also provide a noted synergistic effect.[7]

## Discussion

GC-MS analysis showed that cumin extract contained 61.65% *cuminaldehyde*. Abbaszadegan[9] (2016) also found that the major component of cumin extract was *cuminaldehyde*, but the proportion was 23.18%. In contrast, Naeini et al. (2014) found that the main components of cumin extract were *α-pinene*, *limonene*, *1,8-cineole*, *linalool*, *linalyl acetate*, and *α-terpinol*.[7] Differences in the composition of cumin extract may be due to differences in weather, season, geographic location, and geological composition of soil for cumin planting.[10,11] The antimicrobial activity of cumin

This in vitro research was conducted with *C. albicans* ATCC 10231 samples because it is commonly used to test the effects of an antifungal material.[12]

This fungal strain forms biofilms and has been used extensively as a representative strain for clinical and laboratory research. According to Tables 2 and 3, cumin extract had an antifungal effect on planktonic *C. albicans*. Concentrations of 0.4 µL/mL produced the strongest antifungal effects than those at 0.1, 0.2, and 1.6 µL/mL. This result is in accordance with the results of a study conducted by Kamble (2015) which cumin extract was effective in inhibiting the growth of *C. albicans* at a concentration of 0.4 µL/mL.[13]

In the antifungal effect test on the *C. albicans* biofilm group the lowest OD values were in the cumin extract group of 0.1 µl/ml concentration (In Table 2). However, there was no statistically significant difference between test groups.

In the comparison of the effects of cumin extract between planktonic and biofilm groups, we found that OD was the lowest at a cumin extract concentration of 0.4 µL/mL ($P < 0.05$), suggesting that the antifungal effect of cumin extract at 0.4 µL/mL was better against planktonic C. albicans than against the biofilm form. This may be attributed to the fact that after 48 h, the biofilm forms an extracellular matrix that protects the fungus from the antifungal agent, thereby conferring more resistance. In contrast, the antifungal effect on planktonic *C. albicans* was greater because the cumin extract could contact the fungus directly without being hindered by the extracellular matrix layer.

Gulati and Nobile (2016), stated that the more mature a biofilm is, the greater its resistance to antimicrobial agents and the immune system.[14] This is influenced by the complexity of biofilm architecture, biofilm matrix, and increased expression of drug efflux pumps

and metabolic plasticity. Biofilm maturation produces a complex layer; composed of polymorphic cells (hyphae, pseudohyphae, and round cells) and coated by a thick extracellular matrix and provides protection against both chemical and physical injuries.

Cumin extract has the ability to penetrate fatty acyl chains that form the lipid bilayer of fungal membranes. This causes the membrane permeability and function to change, thereby affecting regulation and membrane function that can alter cell wall synthesis, growth, and morphogenesis. The *terpene* component of cumin extract has the ability to inhibit candidal respiration and interfere with mitochondrial activity, causing cell death and other morphological changes.[7]

The results of GC-MS analysis of cumin extract indicated that *cuminaldehyde* to be a major element. Thus, the active substance that acts as an antifungal agent in cumin extract may be *cuminaldehyde*. In accordance with the research finding of Minooeianhaghighi et al.[15] (2016), the main element of *cuminaldehyde* extract (18.8%) is effective as an antifungal agent. In another study, Kedia et al. (2014) stated that *cuminaldehyde* has an antifungal effect on *Aspergillus flavus*.[16]

## Conclusions

Cumin extract has an antifungal effect against the planktonic and biofilm forms of *C. Albicans* ATCC 10231. The antifungal effect of cumin extract is better against planktonic *C. albicans* ATCC 10231 than against its biofilm form.

## Declaration of Interest

The authors report no conflict of interest. This research is approved and funded by HIBAH PITTA from directorate research and community engagement Universitas Indonesia. The publication of this manuscript is supported by Universitas Indonesia.

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