

ANTIFUNGAL EFFECTS OF *ALOE VERA* IRRIGANT ON *CANDIDA ALBICANS* BIOFILM

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ABSTRACT

Objective: *Candida albicans* tolerates unfavorable environmental conditions by forming biofilms, which can cause problems following endodontic treatments. *C. albicans* is the most common fungus found in failed root canals. Using natural irrigants with good antifungal properties for use as synthetic irrigant alternatives during root canal cleaning and shaping is very important. This study aimed to analyze the effects of *Aloe vera* on *C. albicans* biofilm.

Methods: *C. albicans* biofilm was divided into the following five groups: Group I, no treatment (control group); Groups II, III, and IV, *A. vera* with a concentration of 100%, 75%, and 50%, respectively; and Group V, Ethylenediaminetetraacetic acid (EDTA) 17%.

Results: The average biofilm colony count for *C. albicans* for the *A. vera* 100%, 75%, and 50% groups was higher than that for the EDTA 17% group but lower than that for the control group.

Conclusion: *A. vera* has antifungal effects on *C. albicans* biofilm, with the greatest effect occurring at 75% concentration.

Keywords: *Candida albicans*, Biofilm, *Aloe vera*.

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INTRODUCTION

Candida albicans is a common fungus that is present in 3–18% of failed root canal cases [1,2]. Waltimo *et al.* noted that microorganisms such as fungi that are resistant to endodontic treatment can cause secondary infections [3]. Of 692 cases of the failed root canal, 47 (7%) were fungi-related and 48 species of the fungus were identified. Of these, *C. albicans* was the most common fungus found. Sureshchandra and Arun have revealed that *C. albicans* is a resistant microorganism, has dentinophilic properties, and shows affinity on the smear layer [4]. In addition, Sureshchandra and Arun. have revealed that *C. albicans* can survive in the root canal even after root canal cleaning and shaping [4].

C. albicans has several strategies for tolerating unfavorable environmental conditions, such as by altering its morphology from yeast to hyphae, immunomodulating the host defense system, and adapting to any pH environment. In addition, *C. albicans* can form biofilms, perform thigmotropism (contact sensing) to penetrate dentine tubules, switch phenotypes, and produce proteolytic enzymes [1,3,5].

The success of endodontic treatment depends on the elimination of microorganisms such as *C. albicans* from the root canal system. Grossman has highlighted the need to eliminate such organisms to maintain the periapical tissue normal or restore periapical tissue to a healthy stage after root canal [1,4,5]. Thus, the use of an irrigant with good antifungal properties is very important during root canal cleaning and shaping. A study by Ghogre has shown that ethylenediaminetetraacetic acid (EDTA) 17% has better antifungal properties than NaOCl 5% and Savrolin (1.5% CHX and 15% Cetrimide). The antifungal potential of EDTA 17% is due to its ability to dissolve the calcium ions involved in the morphogenesis and pathogenesis of *C. albicans*. The expression of hyphal wall protein 1 (HWP1), a *C. albicans* gene associated with the formation of biofilm, was successfully reduced by EDTA [1,5,6].

The use of natural, rather than synthetic, ingredients to kill fungi is of interest because natural ingredients are more acceptable, their raw materials are easier to obtain, they have fewer side effects, and their

costs are lower [7,8]. Agarry *et al.* have reported that *A. vera*, both in gel and leaf forms, inhibits the growth of *C. albicans*. This finding is supported by a study by Fitriani showing that *A. vera* 25% is adequate for inhibiting the growth of *C. albicans* planktonic type bacteria and that *A. vera* extract 75% is adequate for eliminating *C. albicans* [3-5,9-11]. However, comparative studies of EDTA 17% and *A. vera* as irrigants have not been conducted. Thus, this study aimed to produce an effective irrigant against the most commonly found fungus *C. albicans* in failed root canal treatment.

METHODS

This study was conducted at the Oral Biology Laboratory, Oral Biology Department, Faculty of Dentistry, Universitas Indonesia. The effectiveness of irrigants on *C. albicans* biofilms was assessed by calculating the colony-forming units (CFUs). *C. albicans* used in this study was a pure strain of American Type Culture Collection 10231™. The use of seed stock minimizes the subculture method and has good protective and storage protocols for maintaining the safety and effectiveness of the culture [12]. *C. albicans* ATCC 10231 is a purified *C. albicans* laboratory subculture that can grow as a biofilm and has been widely used as a representative strain in clinical and laboratory studies [10,13,14].

C. albicans ATCC 10231 was swabbed on Sabouraud Dextrose Agar (SDA) media until blended and then incubated for 24 h at 37°C. After culture, *C. albicans* was obtained using needles up to one full loop, inserted into a tube containing 10 ml of Sabouraud Dextrose broth solution, and incubated for 24 h at 37°C. To produce biofilm, 10 µl of *C. albicans* was prepared by adding 10 µl of Sabouraud Dextrose broth and then incubating at 37°C for 24 h. To obtain 100% *A. vera* solution, 5 g of dried *A. vera* extract was placed in a 50-ml flask with 50-ml aquadest. To obtain 75% *A. vera* solution, 7.5 ml of 100% *A. vera* solution was pipetted into a 10-ml flask with 10-ml aquadest. To obtain 50% *A. vera* solution, 5 ml of 100% *A. vera* solution was pipetted into a 10-ml flask with 10-ml aquadest.

C. albicans biofilm was obtained using a micropipette until it ran out in each well. Subsequently, 200 µl of the test material was applied to each

well, except in the control, and allowed to sit for 1 min. Next, 100 µl of phosphate buffer saline (PBS) was applied to each well. As much as, 10 µl was transferred to a microtube and 990 µl of PBS was added to obtain 10⁻² dilutions. Of the 10⁻² dilutions, 10 µl was taken and mixed with 990 µl of PBS to obtain 10⁻⁴ dilutions. Of the 10⁻⁴ dilutions, 10 µl was taken and mixed with 990 µl of PBS to obtain 10⁻⁶ dilutions. The 10⁻⁴ and 10⁻⁶ dilutions were then swabbed in each SDA medium coded according to the well design and incubated for 24 h at 37°C.

Statistical analysis was conducted using SPSS 20.0 software. Shapiro-Wilk test demonstrated that data were normal, and one-way analysis of variance was used to assess differences among the groups. Tamhane's test was used for *post hoc* comparisons when data were not normally distributed. The level of statistical significance was set at p<0.05.

RESULTS

Table 1 shows that the mean *C. albicans* biofilms colony count was the lowest for EDTA 17% (160.67 CFU/mL) and the highest for the untreated group (1852.67 CFU/mL). The mean values of the *C. albicans* biofilm colony count were lower for *A. vera* 100%, 75%, and 50% groups than for the untreated group.

A. vera 75% showed a mean colony count of 863±±176.510 (mean±SD), which was the lowest among *A. vera* 100%, 75%, and 50%, whereas *A. vera* 50% showed a lower mean colony count than *A. vera* 100%. This indicates that *A. vera* 75% (863 CFU/mL) had the best effects of all the concentrations tested.

In Table 2, *A. vera* 75% (p=0.015), EDTA 17% without treatment (p=0.000), *A. vera* 100%, EDTA 17% (p=0.004), *A. vera* 50%, and EDTA 17% groups showed a significant change. These findings suggest an antifungal effect of *A. vera* 75% and EDTA 17% and a better antifungal effect of EDTA 17% than *A. vera* 100% and 50%.

DISCUSSION

This experimental study was aimed at observing the antifungal properties of *A. vera* as a new irrigant with antifungal potential against *C. albicans* biofilm.

Here, an antifungal efficacy test was conducted on *C. albicans* biofilm because biofilms are often found in teeth with persistent root canal infection, which is indicated for root canal treatment. According to Waltomi *et al.*, *C. albicans* in root canal cases is present in the biofilm form; therefore, the evaluation of the effect of antifungal irrigant on *C. albicans* biofilm can better describe the conditions in root canals than described by bacterial culture studies of the planktonic form [3]. Fungi in biofilm form are more protected, particularly if there are

smear layers, and can be more difficult to eliminate than planktonic fungi [15,16]. The differences in resistance to antifungal agents are much greater in species that are present in mature biofilms than similar species growing in the planktonic form. This is due to the virulence of *C. albicans*. Further, the hyphae were only found in *C. albicans* biofilm. In this study, biofilm was formed in a 96-well plate to allow its growth on standardized surfaces and incubated for 24 h because it has been estimated that *C. albicans* biofilms are formed within 24 h, thus providing a more accurate assessment of the effectiveness of antifungal agents [17]. It was important to standardize the age of biofilms when comparing the effectiveness of antifungal agents [15].

A. vera has the ability to inhibit the growth of *C. albicans*, as supported by Sujatha *et al.* [16]. Components such as aloin, acemannan, and other ingredients found in *A. vera* play an important role as antifungals and immunomodulators that synergistically work, providing a promising outcome for the development of *A. vera* as an alternative irrigant in endodontic treatment. The selection of 100%, 75%, and 50% concentrations of *A. vera* was based on previous studies that examined the effectiveness of *A. vera* on planktonic *C. albicans*.

Our results revealed that *A. vera* 75% has better antifungal properties than *A. vera* 100% and 50%. This may be because 98–99% of the content of *A. vera* is water and the water content is still high at 100%; thus, the mechanism of action of acemannan and aloin may not be optimally working. This finding contradicts with a previous study which found that a higher concentration of *A. vera* exhibits antifungal properties. This may be due to differences in the solution used to make *A. vera* extract. The previous study used ethanol, whereas aquadest was used in this study, which was also used by Fitriani [6,11]. Our findings are consistent with those previous studies which demonstrated the effectiveness of *A. vera* against *C. albicans* [6,16].

Finally, both EDTA 17% and *A. vera* 75% showed statistically insignificant results; therefore, it was concluded that EDTA 17% and *A. vera* 75% had the same antifungal potencies. EDTA 17% has an antifungal effect through its interaction in the cell walls of *C. albicans* that inhibits the growth and affects the nutritional condition of *C. albicans*. In addition, it can decrease the expression of the HWP gene and hyphae marker genes, which play an important roles in the formation of biofilm. Sen *et al.* have shown that EDTA 17% has a better antifungal potency as an irrigant against *C. albicans* than CHX, MTAD, and sodium hypochlorite 2.52% [2,9,14].

CONCLUSION

A. vera showed an antifungal effect on *C. albicans* biofilm. Further, *A. vera* 75% had a better antifungal effect than *A. vera* 100% and 50%.

Table 1: Mean *C. albicans* biofilm colony count (in CFU/mL) after exposure to the antifungal irrigant

Test material (%)	n	Mean colony count±SD	p-value
<i>A. vera</i> 100	3	1374±571.982	0.000*
<i>A. vera</i> 75	3	863±176.510	
<i>A. vera</i> 50	3	1062±170.666	
EDTA 17	3	160.67±8.963	
<i>C. albicans</i> biofilm without treatment	3	1852.67±90.163	

*p<0.05, *C. albicans*: *Candida albicans*, *A. vera*: *Aloe vera*, CFU: Colony-forming unit

Table 2: Significance values of fungal colony quantities in *C. albicans* biofilm across groups

Test material (%)	<i>C. albicans</i> biofilm without treatment	<i>A. vera</i> 100%	<i>A. vera</i> 75%	<i>A. vera</i> 50%	EDTA 17%
<i>C. albicans</i> biofilm without treatment	-	0.638	0.015*	0.063	0.000
<i>A. vera</i> 100	0.638	-	0.503	1.000	0.004*
<i>A. vera</i> 75	0.015	0.503	-	1.000	0.121
<i>A. vera</i> 50	0.063	1.000	1.000	-	0.028*
EDTA 17	0.000	0.004*	0.121	0.028*	-

**post hoc* Tamhane p<0.05, *C. albicans*: *Candida albicans*, *A. vera*: *Aloe vera*

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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