

Investigating the Anti-fungal Activity of Different Concentrations of *Aloe vera* in *Candida albicans* Infection under In Vitro Conditions

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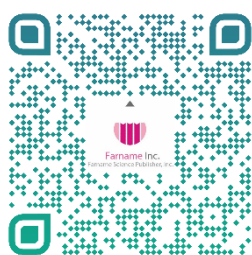
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ABSTRACT

Background & Objective: A variety of synthetic and chemical drugs have been established for the treatment of candidiasis, but each has some limitations and its probable side effects. This study attempts to touch upon the antifungal activity of different concentrations of hydroalcoholic extracts of *Aloe vera* against *Candida albicans* in *in vitro* conditions.

Materials & Methods: Hydroalcoholic extract from *Aloe vera* leaves was tested for anti-fungal activity via an *in vitro* study. Anti-fungal activity and minimum inhibitory concentration (MIC) were determined by the disk diffusion method. *Aloe vera* hydroalcoholic extracts (75%, 50%, 25%, and 12.5%) were used as test groups. The data were analyzed by ANOVA using SPSS 23 software. The level of statistical significance was set at $p \leq 0.05$.

Results: The results revealed that *Aloe vera* contained substantial anti-fungal activity. There was a significant discrepancy in the mean diameter of the inhibition zone of *C. albicans* growth among different concentrations of *Aloe vera* (p -value=0.001). Also, there was a statistically significant difference between the average diameter of the inhibition zone of *C. albicans* growth at a concentration of 12.5% of *Aloe vera* extract compared to concentrations of 25%, 50%, and 75% *Aloe vera*, and concentration of 50% with 25% and 75% extracts. *Aloe vera* extract at 75% concentration effectively inhibited the growth of *C. albicans* compared with the positive control-nystatin. In this study, *Aloe vera* concentration of 20% was determined as the MIC for *C. albicans*.

Conclusion: In adherence to the present results, it seems that *Aloe vera* extract, which is inexpensive and has no side effects, could be introduced as an alternative to nystatin.

Keywords: In Vitro Techniques, Minimal inhibitory concentration, *Aloe*, Antifungal, *Candida albicans*



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Introduction

Among the *Candida* species, *C. albicans* has mainly been proposed to bring about oral candidiasis in immunocompromised individuals. This is attributed to the suppression of local and systemic defense mechanisms (1). It is a natural human oral flora in 20-50% of the healthy individuals that engenders infection in conditions such as weakened immune system (2). The most common local and systemic causes that predispose oral candidiasis subsided to salivary secretion, inappropriate dental prostheses, endocrine disorders such as diabetes, nutritional deficiencies, smoking, poor oral hygiene, use of immunosuppressive drugs, chemotherapy, and radiation therapy (3).

Complications of *Candida* overgrowth in the mouth are local discomfort, altered taste, and dysphagia, which

results in malnutrition, slow recovery, and prolonged hospitalization (4).

Currently, the first choice for the treatment of oral candidiasis is nystatin (5). Bad taste and nausea are among the side effects of this chemical medicine. Furthermore, since nystatin is stable in the form of powder, it must be continuously diluted to use. This drug should be consumed every 6 h for 14-21 days, which is unpleasant for patients (6). Natural products are valuable resources in Persian traditional medicine and have been long used for the treatment of diseases. All over the world, many plants have been exploited for their medicinal value (7). With the current growing interest in the application of scores of herbal-originated medicines, physicians and patients have been persuaded to use herbal instead of chemical

medicines. Progression in this field could improve patient cooperation (8).

Given the side effects of chemical drugs and complex production stages as well as the fact that humans can develop resistance to chemical drugs, many herbal medicines are commonly discussed (3, 9). One of the most widely used plants is *Aloe vera* which has a growing consumption trend. This is a tropical plant grows in most parts of Asia and north Africa with succulent leaves (10), which grows rapidly in tropical regions (3).

Aloe vera has anti-inflammatory, wound healing, scar reduction, antimicrobial, and anti-fungal effects due to its active ingredients (11). *Aloe vera* is also effective in treatments related to gingivitis, periodontal surgery (12), aspirin burns (13), angular cheilitis, aphthous ulcers (10), lichen planus (14), burning mouth syndrome (15), and wound healing (16). The anti-microbial, anti-fungal, and anti-inflammatory effects of *Aloe vera* are well-known worldwide (17). Previous literature has focused on the ethanolic extract of *Aloe vera* (18), whereas this study applied its hydroalcoholic extract. The issue has figured considerably in medicine. This study attempts to determine the antifungal activity of different concentrations of hydroalcoholic extracts of *Aloe vera* against *C. albicans* in *in vitro* conditions.

Materials and Methods

Preparation of inoculums

First, the *C. albicans* strain PTCC 5027 was purchased from Iran Industrial Fungi and Bacteria Collection Center (IROST, Karaj, Iran), and then cultured on Sabouraud dextrose agar medium (Merck, Germany) containing chloramphenicol (50 mg/L). Following that, the media were kept at the ambient temperature for 24h, refrigerated, and applied for drug susceptibility tests (19). *C. albicans* suspension equivalent to 0.5 McFarland (1×10^6 CFU / ml) was prepared in sterile physiological serum using a hematocytometer slide (Neobar slide) method. Disk diffusion and broth microdilution tests were kept at 4°C for susceptibility testing.

Plant material and extraction

We obtained authority to access plant samples, *Aloe vera* leaves were collected and prepared by researchers. The plant of *Aloe vera* (leaves) was harvested from a local farm in the south of Iran. The plant was identified in the Herbarium Research Center of Medicinal Plants. The collected mature and fresh leaves of *Aloe vera* were thoroughly washed with sterile distilled water, and their thick epidermis was then dissected longitudinally into pieces (19). To prepare the hydroalcoholic extract of *Aloe vera*, 200 ml of *Aloe vera* gel was extracted and collected in sterile containers (Pharmaceutics Dep. Pharmacy School, Tehran University of Medical Sciences). The gel was extracted by accelerated maceration with 70% ethanol. The maceration process was carried out for three days with a magnetic stirrer (Labtron, Iran) to gain a hydroalcoholic extract at a ratio of 80:20. The extract was concentrated by a rotary evaporator (Heidolph, Germany)

at 30 °C and dried under a vacuum in a desiccator. The dry weight of the sterile extract was determined using a Sartorius scale (AND, German) with a precision of 0.0001 g, equivalent to 2 g.

Anti-fungal susceptibility testing

Aloe vera and nystatin minimum inhibitory concentration (MIC) as well as their titrations were determined using the disk diffusion method based on the clinical and laboratory standard institute methods (20).

In this study, the anti-fungal activity of *Aloe vera* extracts against *C. albicans* was investigated by two methods: disk diffusion and microdilution (19). This is compatible with Jain et al (21). In this *in vitro* study, different concentrations of hydroalcoholic extracts of *Aloe vera* (12.5%, 25%, 50%, 75%) were evaluated as the test groups, and 100,000 IU concentration of nystatin (Pars Daroo, Iran) was used as the positive control (gold standard), and 1% dimethyl sulfoxide (DMSO) solution (*Aloe vera* extracts' solvent) was applied as the negative control. In the next stage, the purchased sterile blank disks (Iran Laboratory Industries, Iran) were immersed at serial concentrations of *Aloe vera* extracts (12.5%, 25%, 50%, and 75%), and nystatin for 24h and dried in an autoclave at 50 °C.

The susceptibility of *C. albicans* to *Aloe vera* extract and nystatin was measured and compared by disk diffusion method (18). After culturing, disks containing different concentrations of *Aloe vera* extract and nystatin were placed on the culture medium at 4 cm intervals (with 8 replicates for each concentration). The cultures were kept in an incubator at 30 °C for 48 h. The diameter of the inhibition zone of *C. albicans* growth around discs containing different concentrations of *Aloe vera* extract and nystatin was measured using a caliper. The results and the mean diameter of the inhibition zone around each disk were investigated and compared.

Statistical analysis

The data were compiled in MS Office Excel. Statistical analysis was performed using the SPSS version 23 software package (SPSS Statistics for Windows, Version 23.0. Chicago: SPSS Inc.). The MIC of the extract was determined and compared with the MIC of nystatin via running an ANOVA test. Given the test power of 80%, the standard deviation of 1.5, and the significant difference of at least two units in the mean colony count, the number of eight repetitions in *Aloe vera* samples was evaluated. A p-value less than <0.05 was accommodated to be statistically significant. The study design was approved by the Local Medical Ethics Committee of Yazd Shahid Sadoughi University of Medical Sciences (approval ID: IR.SSU.REC1398.016).

Results

To shed light on the anti-fungal activity of different concentrations of *Aloe vera* extract and nystatin on the growth of *C. albicans*, we used the disk diffusion method to determine MIC with 8 replicates for each

concentration. According to the results, and based on the serial concentrations of *Aloe vera* extract (75%, 50%, 25%, 12.5%), the mean diameters of the inhibition zone of *Candida albicans* growth were 7.56 ± 0.49 , 4.81 ± 0.53 , 0.75 ± 0.47 , and 0.00 mm, respectively. As set out in [Table 1](#), a higher concentration of *Aloe vera* extract had a higher anti-fungal activity.

To determine the MIC, we prepared 15% and 20% concentrations of *Aloe vera* solution and conducted eight replications for them. The results of MIC and sensitivity test of disk diffusion displayed that the 15% concentrations of *Aloe vera* had inadequate anti-fungal activity. The 20% concentrations were reflected in the results as the MIC for *C. albicans* in the present study ([Table 1](#)).

Table 1. Zone of growth Inhibition in hydroalcoholic extracts of Aloe vera (mm)

Zone of growth Inhibition of Aloe vera Concentrations	Mean \pm SD	Minimum Zone of growth Inhibition	Maximum Zone of growth Inhibition
(12.5%)	0	0	0
(25%)	0.75 ± 0.46	0	1.5
(50%)	4.81 ± 0.53	4	5.5
(75%)	7.56 ± 0.49	7	8

P-value= 0.001
Kruskal-Wallis test

Overall, the results presented below indicate that the mean diameter of the inhibition zone of the *C.albicans* growth beard was significantly different for various

concentrations of *Aloe vera* ($p=0.001$). The zone of inhibition of negative and positive control groups are set out in [Figure 1](#) and [Table 2](#).

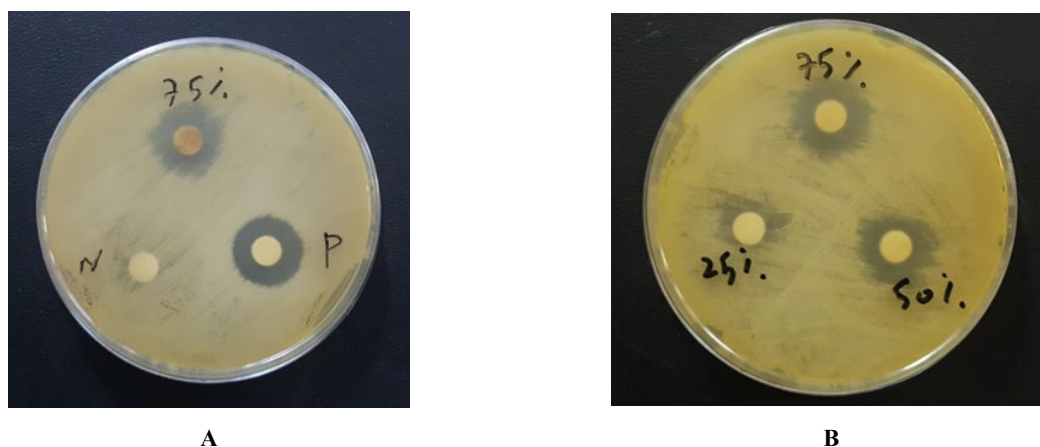


Figure 1. Zone of Inhibition of negative control (DMSO 1%) and positive control (Nystatin) and 75% concentration of Aloe vera (A) in comparison with 25%, 50% and 75% concentration of Aloe vera (B)

Table 2. Zone of Inhibition in hydro-alcoholic extracts of Aloe vera (mm), negative control (DMSO 1%) and positive control (Nystatin) at different concentration by Disc diffusion method

Zone of Inhibition groups	Mean \pm SD	Minimum Zone of Inhibition	Maximum Zone of Inhibition
<i>Aloe vera</i> Concentration (12.5%)	0 ± 0	0	0
<i>Aloe vera</i> Concentration (15%)	0.06 ± 0.17	0.5	0
<i>Aloe vera</i> Concentration (20%)	0.68 ± 0.45	1	0
positive control (Nystatin)	9 ± 0.46	8.5	10

Zone of Inhibition groups	Mean ± SD	Minimum Zone of Inhibition	Maximum Zone of Inhibition
100000 IU			
negative control (DMSO 1%)	0	0	0

P-value= 0.008
Mann-Whitney Test

As can be observed from [Figure 1](#), the maximum diameter of the inhibition zone of *Albicans* growth was measured in nystatin, and 75% *Aloe vera* extract, respectively. The mean diameter of the inhibition zone of *C. albicans* growth was statistically significant at concentrations of 15% and 20% *Aloe vera* extract, confirming with DMSO 1% did not affect *Candida* growth ([Table 2](#)).

To determine the MIC of nystatin, and reach a gold standard, serial dilutions of $\frac{1}{2048}$, $\frac{1}{1024}$, $\frac{1}{512}$, $\frac{1}{128}$, $\frac{1}{64}$, $\frac{1}{32}$, $\frac{1}{16}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1 were prepared. Three replicates were performed at each dilution, and the nystatin MIC, and inhibition zone of *C. albicans* growth diameter was determined as $\frac{1}{1024}$ and 1.3 mm in three replicates, respectively ([Table 3](#)).

Table 3. Susceptibility profile of Nystatin against *C. albicans*

Nystatin dilution Zone of Inhibition	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$	$\frac{1}{2048}$
Replication 1	9	8.5	7.5	7	6.5	6	5.1	0.9	3.1	3	1.4	0
Replication 2	9	8	8	7	7	7	4.6	4.1	3.5	2.9	1.6	0.5
Replication3	8.5	7.5	7	7	6	6	4.8	4.2	3.6	2.5	1.1	0
mean	8.8	8.7	7.5	7	6.5	6.3	4.8	4	3.4	2.8	1.3	0.1

Discussion

The production of synthetic drugs could improve health care facilities worldwide. The present study indicates that the *Aloe vera* extract has anti-fungal activity. Saniasiaya et al. (22) and Shireen et al. (23) concur the anti-fungal activity of *Aloe vera* extract increases at higher doses. This is also in agreement with studies done by Shireen et al. (23).

This study probe highlights the fact that *Aloe vera* extract is effective in the case of candidiasis, especially for immunocompromised patients like HIV subjects and organ transplant patients, as use decrease the burden of the medication. Owing to the fact that it is relatively easy to cultivate, the usage of *Aloe vera* is also cost-effective.

Given the evident treatment applications of herbal medicine, returning to nature and reusing herbal medicines can prevent the probable side effects of chemical drugs (24). Iran has a rich and valuable herbal resource due to its diverse climatic conditions and nature. One of the most valuable medicinal plants is *Aloe vera* (17). According to the above-mentioned functional effects of *Aloe vera*, this survey investigated the anti-fungal activity of various concentrations of *Aloe vera* extract on *C. albicans* through an *in vitro* study.

In addition to the anti-fungal impact of *Aloe vera*, other potential areas of application in dentistry are applications directly at sites of periodontal surgery (25), aspirin burns (26), Angular cheilitis, aphthous ulcer. *Aloe vera* could also be used to treat oral lichen planus, and alveolar osteitis (23).

In the current study, the mean diameter of the inhibition zone of *C.albicans* growth at 75 % concentration extract of *Aloe vera* was comparable to standard nystatin suspension and 20% *Aloe vera* concentration was determined as the MIC for *C. albicans*.

In the present study, we used the hydro-alcoholic extracts of *Aloe vera* and the disk diffusion method to evaluate the anti-fungal activity of *Aloe vera* extracts, which is in line with previous literature (21, 23, 27).

In the present study, serial concentrations of the hydroalcoholic extract of *Aloe vera* (12.5%, 25%, 50%, 75%) were examined, and our experiments are in line with Jain et al.(21) Abdul-Jabbar et al.(28) also used hydroalcoholic extracts of *Aloe vera* at concentrations of 15%, 20%, 25%, and 30%, which was at variance with this study. It was mentioned *Aloe vera* extracts at high concentration had a reliable anti-fungal activity. Of course, the aqueous extract of *Aloe vera* was less

effective than the alcoholic type. Since the nature of the anti-fungal activity of alcohol is more than water (29), this study applied hydroalcoholic extracts of *Aloe vera*, similar to the previous literature (30).

Nystatin (100,000 IU) was used as a positive control and the gold standard for comparing *Aloe vera*'s anti-fungal properties, which is in alignment with Al-Husseini (2010), Stanley et al. and Amjed et al. (2017) studies (27, 31, 32). Chlorhexidine and Amphotericin B were used as a positive control by Shireen et al. (2017) which is congruous with our study (23). In good agreement with previous studies, 1% DMSO was applied as the negative control group (33, 34).

In the present study, the mean inhibition zone of growth *C. albicans* was measured as zero, 0.75 ± 0.46 , 4.81 ± 0.53 , and 7.56 ± 0.49 mm at 12.5%, 25%, 50%, and 75% concentrations of *Aloe vera* extract in eight replications, respectively.

Jain et al. (21) also evaluated 12.5%, 25%, 50%, and 100% *Aloe vera* concentrations and reported zones of inhibition of 0, 0, 1.06 ± 0.41 , and 3.35 ± 0.59 mm, respectively. This is lower than the rate obtained in the present study. This is why they used only the solid mucilaginous gel of *Aloe vera* instead of leaf extracts (as were used in the present study). Based on their results, concentrations of 50% and 100% of *Aloe vera* had anti-fungal effects, which is in agreement with our findings. In this regard, our findings are in line with those of Jain et al. (21). A probable mechanism of the gained antifungal properties is attributed to the tannins, saponins flavonoids, alkaloids, and steroids, contained in *Aloe vera* extracts (18).

Since the results of an experiment can be affected by various factors such as essential oil extraction method, inoculation volume, growth phase, the medium used for growth, ambient pH, incubation time, and incubation temperature, comparing the published data are controversial and difficult to generalize. Many studies to date, however, have attempted to cite MIC as a criterion for determining the antimicrobial activity of essential oils (35, 36).

Our finding is at variance with the research of Jain et al. (21), as we elicited 20% of *Aloe vera* extract as MIC, while previous evidence reported 15% (21). The susceptibility method is probably responsible for this discrepancy. Jain et al. used a broth microdilution test for MIC determination while the disk diffusion method was applied in the present study (21).

The findings provide support for the anti-fungal activity of *Aloe vera* extract. However, our research suffers a critical limitation; due to the restricted availability of the hydroalcoholic extract of *Aloe vera* to prepare herbal extracts at different concentrations.

Given that our findings are based on some limitations, the results of our analyses should be interpreted with the utmost caution.

Modalities targeted at the use of *Aloe vera* against *C. albicans* can prove to be more useful and accordant compared to common anti-fungal drugs for therapeutic goals against a variety of oral fungal diseases (18). There are further research agendas here that could fruitfully be perused. For example, the antifungal activity of *Aloe vera* can be increased in many folds in combination with other herbal medicines. Hence, it would be further evaluated. The conclusion of this survey should be interpreted with caution. More studies should be programmed in the field of phytochemistry exercising attention to both *in vivo*, and *in vitro* analysis. The isolation of bioactive compounds could be a good idea for future studies.

Conclusion

The extract of *Aloe vera* has concentration-dependent anti-fungal activity against *C. albicans*. There was a significant difference in the mean diameters of the non-growth zones based on the concentration of *Aloe vera*. *Aloe vera* extract at a 75% concentration effectively inhibited the growth of *C. albicans*. A concentration of 20% was determined as the minimum inhibitory concentration for *C. albicans*. Thus, *Aloe vera* can be proposed as an alternative traditional anti-fungal choice to therapeutic goals.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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