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ORIGINAL RESEARCH

A Comparative Laboratory Study on the Effectiveness of Aloe vera Extracts and Nystatin in Inhibiting the Growth of Candida albicans

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Abstract

Background: Candida albicans is the most prevalent cause of oral fungal infections. This study aimed to compare the effectiveness of different dilutions of Aloe vera extracts (water and alcoholic)in inhibiting the growth of oral Candida albicans compared to nystatin.

Materials & Methods: This study was an experimental and laboratory investigation of a common strain of Candida albicans species in Iran. Different dilutions (1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 mg/ml) of alcoholic and water extracts of Aloe vera and nystatin were prepared. The study measured the diameter of the inhibition zone of Candida albicans and assessed the impact of nystatin or Aloe vera extracts on this parameter. All data were analyzed using two-way ANOVA, Post hoc Bonferroni test and t-test (α=0.05).

Results: Both water and alcoholic extracts of Aloe vera at concentration of 200 mg/ml produced a larger non-growth halo. Water extracts were more effective than alcoholic extracts at certain dilutions ($p<0.001$). Both water and alcoholic Aloe vera extract dilutions showed better results than nystatin ($p<0.001$).

Conclusion: The antifungal efficacy of water-soluble Aloe vera was significantly superior to alcoholic extracts and nystatin.

Keywords: Aloe vera; Nystatin; Candida albicans

Introduction

Oral candidiasis is the most prevalent opportunistic infection affecting the oral mucosa, resulting from the overgrowth of Candida in the oral cavity (1, 2). Candida albicans is the predominant causative agent of oral candidiasis. It is commonly found in the mouths of 20 to 50% of healthy individuals, found on all oral mucosal surfaces. However, when local or systemic predisposing factors are present, it transforms into a pathogenic agent (2).

Nystatin mouthwash is the standard topical treatment used for fungal infections (2). This antifungal medication was initially derived from Streptomyces noursei and is widely used due to its limited absorption through the digestive system. Its antifungal properties arise from its ability to bind to membrane sterols, which impacts the permeability of fungal membranes. Although this poly N antibiotic extensively used against fungal pathogens, patient dissatisfaction is often associated with nystatin mouthwash due to its bitter taste, its usage(for four times daily), and the need for repeated preparation in the form of a suspension (2-4). Considering the costs, side effects, and the growing resistance to available antifungal drugs on the market, there is an increasing interest in exploring natural herbal remedies for fungal infections. Traditionally used medicinal plants, represent a valuable source of antimicrobial agents and are readily accessible, especially in rural areas. Furthermore, they are more cost-effective than modern pharmaceutical drugs. Secondary metabolites produced by medicinal plants serve as a crucial source of numerous pharmaceutical agents (5).

Aloe vera, a member of the Liliaceae family, is one of the most widely utilized plants in the pharmaceutical, cosmetic, and nutritional industries (6, 7). The Aloe vera plant is rich in biologically active compounds. Fresh Aloe vera leaves consist of 99.5% water and approximately 75 different compounds, including vitamins, minerals,

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enzymes, sugars, anthraquinones, lignin, tannic acids, polysaccharides, glycoproteins, saponins, sterols, amino acids, and salicylic acid (6, 8). Aloe vera-derived glycoproteins exhibit anti-inflammatory and antimicrobial effects. Concentrated Aloe vera extract has demonstrated antimicrobial effectiveness in treating conditions such as typhoid and Newcastle disease in chickens. Moreover, the hydroalcoholic extract from fresh Aloe vera leaves has displayed antifungal properties against various fungal species (9). Prior research by Bajwa et al (6) and Nabigol and Asghari (7) have highlighted the effects of Aloe vera extract on Aspergillus and Penicillium strain. In the Das et al. (8) study, a 14-kilodalton protein was isolated from Aloe vera extract, revealing antifungal and anti-inflammatory properties against Candida strains. Various studies have provided evidence demonstrating that Aloe vera extract hinders the growth of Candida albicans in a laboratory setting (9-12).

Given the limited research performed in this field, our study aimed to compare the impact of Aloe vera plant extract on the growth of oral Candida albicans compared to nystatin.

Materials and Methods

This study was conducted as an experimental laboratory at the Oral Medicine Department, Isfahan Azad Dental School. This research was approved by the University's ethical committee (1402-04-14-21730). A botanist collected and prepared the Aloe vera samples. A common Candida albicans samples in Iran (PTCC5027) were sourced from the Iranian Practical and Industrial Research Organization.

Initially, Aloe vera leaves were dried and processed into a uniform powder using an electric mill. Next 200 grams of this powder was measured using a digital scale and placed into a beaker. Distilled water (1000 ml) was then added to the powder, and the mixture was heated for 10 minutes. After heating the beaker was then sealed with aluminum foil for 40 hours. Finally the mixture was filtered followed by filtration using filter paper to obtain a 20% water extract through a Bain-Marie process (13).To create the alcoholic extract, 200 grams of Aloe vera powder were completely immersed in 1000 ml of 96% ethanol. The Erlenmeyer flask was sealed with aluminum foil, placed on a shaker set to 90 rpm for 48 hours. After this process the solution was filtered using filter paper and subjected to vacuum distillation to separate the solvent from the extract. This resulted in a pure alcoholic extract with a concentration of 20%, which was stored in a refrigerator for microbial testing (14).

Hypothetical dilutions of Aloe vera were selected to encompass a wide range of concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 mg/ml). These dilutions were prepared by using eight sterile microplates, each containing 100 ml of Muller-Hinton broth culture medium. The Aloe vera extracts were then diluted at a ratio of 400 mg/ml with a 2% DMSO (dimethyl sulfoxide) solution from Cenavis, Spain. The process begun with adding 100 microliters to the first well, followed by transferring 100 microliters from each well to the next one, and discarding 100 microliters from the last tube. This process was repeated ten times (15).

The nystatin dilution was prepared following the Sigma factory protocol, utilizing 100,000 units of nystatin powder. Serial dilutions were prepared in the same manner as for the extracts, maintaining a fixed nystatin concentration.

A culture medium was prepared by adding 1.2 gram of culture medium powder to 100 cc of distilled water, which was then sterilized in an autoclave at 121°C for 15 minutes. After cooling, this medium was utilized for microdilution in a microplate.

To prepare a 0.5 McFarland standard, 1% sulfuric acid was first made using 98% sulfuric acid. This was accomplished by mixing 99 cc of distilled water with 1 cc of 98% sulfuric acid (caution was exercised when adding the acid to the water due to the exothermic nature of the reaction). Subsequently, 1.175 grams of barium chloride powder was added to 100 cc of distilled water, creating a 1.175% barium chloride solution. Then, 99 cc of 1% sulfuric acid was mixed with 0.2 cc of barium chloride. This resulting mixture, due to the precipitation of barite (barium sulfate) $BaSO_4$, displayed turbidity, comparable to McFarland's 0.5 standard. This visual comparison was conducted in adequate lighting conditions. McFarland's 0.5 suspension had an optical absorption of 0.08-0.1 at a wavelength of 625 nm (14).

First, the Candida cultures were applied to Muller-Hinton Agar culture medium using a sterile syringe, then the plate was incubated for 24 hours in a 37-degree Celsius incubator, and after 24 hours, the formation of Candida colonies and their appearance were checked.

A suspension of Candida albicans was prepared using 24-hour cultures grown on Muller-Hinton agar medium at 37°C. Sterile physiological saline was used to create a uniform suspension with consistent fungal concentrations. The turbidity of this suspension was assessed using the McFarland standard which

corresponds to a grade of 0.5. The prepared fungal suspension was diluted with distilled water at a 1:20 ratio. Subsequently, 10 microliters of the diluted fungal suspension were added to the wells and incubated at 37 degrees Celsius for 24 hours, resulting in a microbial suspension of $5x10^4$ CFU/ml in each well.

Due to the turbidity of the extract, it was not visually detectable. Therefore, turbidity measurements had to be read using an optical density (OD) measurement in an ELISA Reader, or samples were needed to be cultured from each well using a sterile swab onto Mueller Hinton agar culture medium. The latter method was chosen. After incubation at 37°C for 24 hours, the formation or absence of colonies was assessed directly and compared with positive and negative control plates (15). For control purposes, both a positive control and a negative control were utilized. Muller-Hinton and Candida culture medium served as the positive control, while culture medium and extract were used for the negative control. 10 sample were selected for each dilution in 3 plates (nystatin, water and alcoholic extracts).

Results

The mean and standard deviation of inhibition halo for Candida albicans by three groups (water extracted Aloe vera, alcoholic extracted Aloe vera and nystatin) were presented in table 1 and figure 1.

Table 1. The mean and standard deviation of inhibition halo for three studied groups

Concentration (mg/ml)	Aloe vera alcoholic extract Mean ± SD (mm)	Aloe vera alcoholic extract Mean ± SD (mm)	Nystatin Mean± SD (mm)
200	11.52±0.39	10.34±0.39	2.8±0.59
100	10.36±0.56	9.63±0.68	2.97±0.57
50	9.37±0.58	8.48±0.53	3.06±0.62
25	8.37±0.57	7.63±0.6	3.03±0.6
12.5	7.29±0.53	6.55±0.96	2.85±0.57
6.25	6.48±0.6	5.9±0.76	3.02±0.51
3.12	5.73±0.55	5.23±0.82	2.65±0.46
1.56	4.87±0.44	4.52±0.89	2.88±0.63

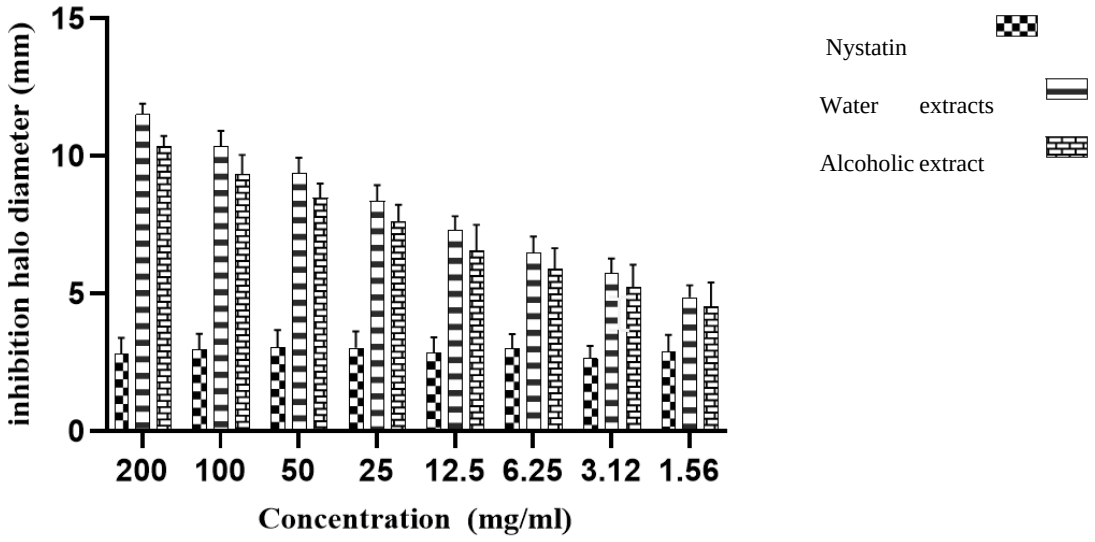

Figure 1. The mean and standard deviation for three studied groups (Aloe vera water and alcoholic extract and nystatin).

The largest inhibitory halo diameter was observed at a highest concentration of 200 mg/ml, while the smallest inhibitory diameter was at lowest concentration (1.56 mg/ml).

Difference between alcoholic and water extract Aloe vera was meaningful for extract ($p<0.001$) and different solution ($p<001$) by using 2-way ANNOVA.

The Post hoc Bonferroni test indicated that the differences in the two-by-two comparisons among the 200, 100, 50, and 25 dilutions for water extracts were statistically significant. However, for the alcoholic extracts, only some concentrations showed significant differences. Comparing the two extracts at different concentrations, the differences were statistically significant at 50 ($p=0.044$), 25 ($p=0.01$), 12.5 ($p=0.01$), 6.25 ($p=0.002$), 3.12 ($p=0.011$),1.56 ($p<0.001$) by using Post hoc Bonferroni test. However, there was no significant difference in the diameter of the growth halo between the water and alcoholic extracts for at concentrations of 100 and 200.

The t-test showed the difference between water and alcoholic extracts with nystatin in different concentrations were statistically significant ($p<0.001$).

Discussion

This study revealed that the water extract of Aloe vera showed a greater diameter of the growth inhibition halo Candida albicans compared to the alcoholic extract. Furthermore, the inhibition halo in both the alcoholic and water extracts of Aloe vera was significantly larger than that of nystatin. This suggests that the antifungal effect of nystatin on Candida albicans is significantly less potent than Aloe vera.

It is noteworthy that Movaghri pour et al. (2), who conducted a laboratory comparison of the antifungal effect of oregano plant extract and nystatin on Candida albicans, reached a different conclusion. They found that nystatin had a more substantial antifungal effect on Candida albicans compared to oregano water and alcoholic extracts. The discrepancy in results between Movaghri pour's study (2) and the present research could be attributed to the different plant species used (oregano vs. Aloe vera), as different plants possess distinct chemical and therapeutic properties. Jain et al. (5) investigated the antifungal activity of various concentrations of Aloe vera gel against oral Candida albicans and found that 100% Aloe vera gel had the most significant effect in reducing Candida Albicans by evaluating zone of inhibition. This aligns with the current research, which demonstrated that the diameter of the inhibition halo increased with higher concentrations of Aloe vera extracts. Shireen et al. (16) also explored the antifungal effect of Aloe vera extract on the inhibition halo of Candida albicans and found that Aloe vera extract exhibited potent antifungal effects, with varying inhibitory effects at different concentrations. The results from the present study are consistent with Shireen findings, as Aloe vera extracts showed a significantly greater effect compared to nystatin. Bernardes et al (9) investigated the impact of fresh Aloe vera leaf extract on reducing the growth of Candida albicans. The results support current research, confirming that Aloe vera extracts have a positive impact on antifungal activity against Candida albicans. Al-Hussaini and Al-Mohana (17) investigated the antifungal effects of the alcoholic extract of Aloe vera against Candida albicans and found that the extract effectively reduced the growth of Candida albicans at different concentrations. This study aligns with the current research. Abdul Wahab and Jasim (18) explored the effects of Aloe vera extract on the inhibition halo of Candida albicans and concluded that Aloe vera extract possesses antifungal properties. This supports the findings in the present research. Abduljabbar et al. (19) studied the antifungal and bacterial effects of the alcoholic extract of Aloe vera and found that higher concentrations of Aloe vera extract exhibited more antifungal activity. While the study differed in terms of which Aloe vera extract (alcoholic vs. water) and water extract was more effective, the observation that increased concentration led to greater antifungal activity corresponds with the results of this research. However, Khaing studied (20) the antifungal and antioxidant effects of Aloe vera leaf extract and reported that the alcoholic extract of Aloe vera had antifungal effects against various fungi but not against Candida albican. This contradicts the findings in the present research, where the alcoholic extract of Aloe vera demonstrated effectiveness in reducing the diameter of the inhibition halo in Candida albicans.

In conclusion, both water and alcoholic Aloe vera extracts were effective against Candida albicans. Water Aloe vera extract was more effective at increasing the diameter of inhibition halo compared to the alcoholic extract and nystatin.

Conflict of interest

No conflict of interest

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