Antifungal Activity of Aloe Vera Leaf and Gel Extracts Against *Candida albicans*: An *In Vitro* Study

M Shilpa¹, Vinaya Bhat², A Veena Shetty³, Mora SR Reddy⁴, Prashant Punde⁵

**ABSTRACT**

**Aim:** The present study was conducted with an aim to assess the antimicrobial activity of ethanolic extracts of aloe vera leaf and gel against *Candida albicans* in vitro.

**Materials and methods:** Fresh leaves were collected from the aloe vera plants naturally grown in Coorg. Aloe vera leaf as well as gel was separated, extracted with 95% ethanol in rotary shaker at constant temperature for 3 days, evaporated in a heating mantle, and stored in screw cap test tubes at 4°C for further analysis. Antifungal activity of aloe vera leaf and gel extracts against *C. albicans* was assessed by well diffusion method. Further, gel extracts of aloe vera at different dilutions (500, 400, 300, and 200 μL) were prepared and the turbidity was analyzed.

**Results:** Results showed that the ethanolic extract of aloe vera leaf did not show antifungal activity against *C. albicans*. A maximum of 99.33% antifungal activity was shown by 400 μL of aloe vera gel extract. The minimum inhibitory concentration of aloe vera gel extract was 200 μL (98.2% inhibition).

**Conclusion:** The ethanolic extract of aloe vera gel showed considerable antifungal activity against *C. albicans*.

**Clinical significance:** Modalities targeted on the use of aloe vera against *C. albicans* can prove to be more beneficial and consistent compared to conventional antifungals for preventive and/or therapeutic purposes against a variety of oral fungal diseases.

**Keywords:** Aloe vera, Antifungal, *Candida albicans*, Natural.


**Introduction**

Natural products are important resources in traditional medicine and have been long used for prevention and treatment of many diseases.¹ All over the globe, many plants have been exploited for their medicinal value. Mukherjee and Wahile reported about the considered opinion of World Health Organization, which states that, “80% of the world’s population are dependent on ancestral medicines for their haleness”. For the healthcare of the remaining 20% population mainly residing in developed countries, therapeutic product of plants plays an important role.²

Aloe vera (Sanskrit- Ghritakumari, Kumara; Hindi- Guarptha, Ghikanvar) a herb, commonly referred to as the “medicinal plant”, is known for its wide range of therapeutic properties. The botanical name of aloe vera is *Aloe barbadensis* Miller and it belongs to lily family. Aloe products are very popular in the market and are widely used in skin care, cosmetics, medical, healthcare, and food industry.³,⁴

Aloe vera is made up of many complex ingredients including polysaccharides, glycoproteins, phenolic compounds, salicylic acid, lignin, hormones, amino acids, vitamins, saponins, and enzymes, which give aloe vera its many beneficial properties including anti-inflammatory, antibacterial, antioxidant, immune-boosting, and hypoglycemic properties.⁵,⁶

Even though several effective antifungal agents are available for oral candida infections, the failure is not uncommon because isolates of *C. albicans* may exhibit resistance to the drug during therapy.⁶

Hence, the present study was conducted with an aim to assess the antimicrobial activity of ethanolic extracts of aloe vera leaf and gel against *C. albicans* in vitro.

**Materials and Methods**

The study protocol was approved by the Institutional Review Board of Coorg Institute of Dental Sciences, Virajpet.

**Preparation of the Ethanolic Extract of Aloe Vera Gel**

Fresh leaves weighing 1 kg were collected from the aloe vera plants (*A. barbadensis* Miller, belonging to Liliaceae family) naturally grown in Coorg. Aloe vera leaf as well as gel was separated, extracted with 95% ethanol in rotary shaker at constant temperature for 3 days, evaporated in a heating mantle, and stored in screw cap test tubes at 4°C for further analysis. Antifungal activity of aloe vera leaf and gel extracts against *C. albicans* was assessed by well diffusion method. Further, gel extracts of aloe vera at different dilutions (500, 400, 300, and 200 μL) were prepared and the turbidity was analyzed.

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in Coorg. After washing the leaves with tap water and then again rinsing with distilled water, the leaves were dissected with a sterile knife into longitudinal sections and gel was scooped out with a sterile sharp spatula, care being taken to avoid the mixture of the fibers into the gel.

Aloe vera gel was macerated in an electric blender to obtain a fine paste (Fig. 1), by adding few drops of 95% ethanol. The macerated gel was equally distributed in conical flasks after which 150 mL of 95% alcohol was added to all the conical flasks. Subsequently, the flasks were kept in rotary shaker for 3 days.

After 3 days, the macerated gel was filtered using Whatman filter paper no. 1. It was then evaporated in a heating mantle and stored in a screw cap test tube at 4°C.

Preparation of the Ethanolic Extract of Aloe Vera Leaf
The leaves which were separated from the gel were washed in distilled water, cut into small pieces with a sterile knife, and kept for drying. After 24 hours, they were pressed with filter paper to remove the moisture content and residual gel of the leaves. Then the leaves were ground into a fine paste using few drops of 95% ethanol.

The ground leaf paste was mixed with 600 mL of 95% ethanol along with constant stirring on a hot water bath at around 45 to 50°C. The mixture was then kept in a magnetic stirrer at a constant temperature of about 38°C for 3 days (Fig. 2) after which it was filtered using Whatman filter paper no. 1. The solution obtained was collected and reduced in the heating mantle to obtain thick dark brownish viscous syrup. This crude ethanolic extract of aloe vera leaf was stored at 4°C for further analysis.

Assessing Antifungal Activity of Aloe Vera Leaf and Gel Extracts by Well Diffusion Method

Preparation of A. albicans Culture
Pure culture of A. albicans ATCC 90028 strain was cultured in Sabouraud dextrose agar (HiMedia laboratories, Mumbai, India) and maintained at 37°C for 24 hours. The 24-hour culture was adjusted to match the 0.5 McFarland turbidity standard to obtain final inoculum of 2 x 10^5 cfu/mL.

Sterile Sabouraud dextrose agar plates were prepared, one each for the leaf and gel of aloe vera, respectively. After swabbing each plate with the suspension of A. albicans, four wells of 8 mm were prepared using a sterile metallic template in both the plates.

Inoculation of the Test media
The leaf extract 500 mg was diluted in 1 mL of dimethyl sulfoxide (DMSO), of which 500 μL of the extract was dispensed into each of the three prepared wells in the first petri plate. Also, 2 g of the gel extract was diluted in 2 mL of DMSO, of which 500 μL of the extract was dispensed into each of the three wells in the second petri plate. The fourth wells in both the plates served as positive control (Fluconazole, 25 μg) and were incubated for 24 hours at 37°C. Hence, both the tests were conducted in triplicates.

After incubation, the zones of inhibition (i.e., locations where no growths of Candida were present) were examined around the wells that contained the test samples. There was no zone of inhibition around the well that contained aloe vera leaf extract samples (Fig. 3). There was a clear zone of inhibition around the well containing aloe vera gel extract samples (Fig. 4). Therefore, microbroth dilution method was used to examine the extent of antifungal activity of aloe vera gel extract against A. albicans.

Assessing Antifungal Activity of Aloe Vera Gel Extract by Microbroth Dilution Method

Preparation of A. albicans Culture
Pure culture of A. albicans ATCC90028 strain was inoculated in yeast nitrogen base (YNB) and incubated overnight at 37°C for 24 hours. It was then centrifuged at 3,000 rpm for 5 minutes at three successive intervals and the supernatant was discarded each time.

The pellet was then resuspended in YNB and the turbidity was adjusted to match the 0.5 McFarland turbidity standard. The McFarland adjusted culture was diluted in 14 mL of Roswell Park Memorial Institute 1640 Medium (RPMI-1640) in a 1:50 ratio.

Preparation of the Test Samples
In a screw cap test tube, 4 g of the aloe vera gel extract was weighed and diluted with 4 mL of DMSO. Gel extracts of different dilutions (500, 400, 300, and 200 μL) were prepared.

Assessing the Antifungal Activity
Subsequently, 500 μL of the RPMI + culture was collected in cuvettes to which gel extracts of different dilutions (500, 400, 300, and 200 μL) were added. The cultures were then incubated for 24 hours. After 24 hours, the biochemical analyzer was used to check the turbidity. Descriptive statistics was used to summarize the results (Table 1).
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**Results**

**Antifungal Activity by Well Diffusion Method (Aloe Vera Leaf and Gel Extracts) and Microbroth Dilution Method (Aloe Vera Gel Extract)**

There was no zone of inhibition around the well that contained aloe vera leaf extract samples (Fig. 3). There was a clear zone of inhibition around the well containing aloe vera gel extract samples (Fig. 4). As shown in Table 2 and Figure 5, the maximum inhibition of 99.33% was shown by 400 μL of the gel extract, while 94.2% of inhibition was shown by 500 μL and 300 μL of the gel extract. From Figure 6 it is clear that, per the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for microbroth dilution, the minimum inhibitory concentration of aloe vera gel extract was 200 μL (98.2% inhibition).

**Discussion**

Among the *Candida* species, *C. albicans* frequently has been reported to cause oral infections in immunocompromised individuals due to suppression of local and systemic defense mechanisms. They can also persist and contaminate the endodontically treated teeth resulting in the failure of treatment. The use of natural products as alternative agents for the control of fungal diseases is considered as an interesting alternative to synthetic fungicides.

Antifungal activity was recorded by the aloe vera gel extract only and not by the leaf extract, against *C. albicans*, in the present

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**Table 1**: Optical densities of the Aloe vera gel extract samples

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Test sample</th>
<th>Dilutions (μL)</th>
<th>Optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>500</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>400</td>
<td>460</td>
</tr>
<tr>
<td>3</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>300</td>
<td>361.5</td>
</tr>
<tr>
<td>4</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>200</td>
<td>364.5</td>
</tr>
<tr>
<td>5</td>
<td>Aloe vera gel extract + RPMI</td>
<td>500</td>
<td>474</td>
</tr>
<tr>
<td>6</td>
<td>Aloe vera gel extract + RPMI</td>
<td>400</td>
<td>451</td>
</tr>
<tr>
<td>7</td>
<td>Aloe vera gel extract + RPMI</td>
<td>300</td>
<td>439</td>
</tr>
<tr>
<td>8</td>
<td>Aloe vera gel extract + RPMI</td>
<td>200</td>
<td>340</td>
</tr>
</tbody>
</table>

(All values are mean of triplicates)

**Table 2**: The inhibition percentage obtained after substituting the above formula with the values obtained for test samples as shown in Table 1

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Test sample</th>
<th>Dilutions (μL)</th>
<th>Growth (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>500</td>
<td>5.8</td>
<td>94.2</td>
</tr>
<tr>
<td>2</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>400</td>
<td>0.67</td>
<td>99.33</td>
</tr>
<tr>
<td>3</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>300</td>
<td>5.8</td>
<td>94.2</td>
</tr>
<tr>
<td>4</td>
<td>Aloe vera gel extract + RPMI + culture</td>
<td>200</td>
<td>1.8</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Hence, inhibition% = 100 – growth%
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In the study by Agarry et al., the growth of *C. albicans* was inhibited by aloe vera leaf but not by the gel.\(^{11}\)

Regarding the antifungal activity of various dilutions of aloe vera gel extract against *C. albicans*, a maximum inhibition of 99.33% was shown by 400 μL of the aloe vera gel extract, while 94.2% of inhibition was shown by 500 and 300 μL of the gel extract. In the study by Tamilarasi et al., 150 μL of the ethanolic extract of aloe vera gel showed the strongest inhibitory activity against *C. albicans* (79%).\(^{12}\)

The degree of inhibition varied depending upon the dilutions of the gel extracts in the present study. In the study by Devi et al. and Renisheya et al., antifungal activity of the aloe vera gel DMSO extracts also varied according to the concentration, with the highest antifungal activity shown by 400 μg/mL, followed by 200 and 100 μg/mL.\(^{13,14}\)

The minimum inhibition concentration that was obtained in the present study was 200 mg/mL (98.2%). This is in contrast to the study by Stanley et al., which revealed that ethanol extract of aloe vera gel was susceptible to *C. albicans*, and the minimum inhibitory concentration of ethanolic extract of aloe vera gel on *C. albicans* was 0.50 mg/mL.\(^{15}\)

It is evident from the present investigation that ethanolic extract of aloe vera gel has antifungal effects against *C. albicans*, which lends more weight to the general acceptability of herbal extracts for therapeutic purposes.

Various studies have been done to assess the antimicrobial activity of aloe vera on *C. albicans*. Aloe vera was observed to have antifungal activity against oral thrush.\(^{16}\) It has also been reported that a processed aloe vera gel preparation inhibited the growth of *C. albicans*.\(^{17}\) Aloe vera leaf extracts have been observed to inhibit the germ tube formation and hence the growth of *C. albicans*. The purified aloe protein has been found to exhibit potent antifungal activity against *Candida paraprilosis*, *Candida krusei*, and *C. albicans*.\(^{18}\)

Previous reports suggest that aloe vera could inhibit infectious diseases by stimulating the host defense mechanism, especially the phagocytic and killing activities of macrophages. Evidence supports the usage of aloe vera as an antimicrobial agent.
and promotes its use in research, especially in toxicology and pharmacology.\textsuperscript{12}

The antimicrobial effect of a dentifrice containing aloe vera has been demonstrated in an in vitro study, in which this phytotherapeutic agent inhibited the growth of diverse oral microorganisms, such as Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, and \textit{C. albicans}.\textsuperscript{19} The results of the present study lend support to the use of the aloe vera gel extracts as home remedies or adding to dentifrices, mouthwashes, and varnishes which may create an oral environment which is unfavorable for \textit{C. albicans}.

It has been reported that aloe vera gel contains glycoprotein with cell proliferating–promoting activity and that it improves wound healing by increasing angiogenesis resulting in increased oxygenation. This is particularly beneficial in patients with sore gums and teeth as well as denture stomatitis.\textsuperscript{5,20}

With the limitation of the study being assessment of antifungal activity using a single extraction method, i.e., ethanolic extract of aloe vera leaf and gel, further studies should be carried out using various extraction procedures along with clinical testing to ascertain the comparative antifungal activity of aloe vera leaf and gel against \textit{C. albicans}.

\textbf{Conclusion}

From the study results, it was concluded that the ethanolic extract of aloe vera gel showed considerable antifungal activity against \textit{C. albicans}. There was varying antifungal activity according to varying dilutions of aloe vera gel extract. The minimum inhibitory concentration of aloe vera gel extract was 200 μL (98.2% inhibition), whereas the maximum of 99.33% antifungal activity was shown by 400 μL of aloe vera gel extract.

\textbf{References}