

Comparison of Antifungal Effect of Aloe vera Gel and Triphala: An *In vitro* Study

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Abstract

Aim: The aim of the present study was to determine and compare the antifungal and inhibitory activities of various concentrations of Aloe vera gel and Triphala against oral *Candida albicans*. **Materials and Methods:** The fungi (*C. albicans*) were isolated from 10 patients with pseudomembranous candidiasis and patients with denture stomatitis and transferred to Sabouraud's broth, which was later incubated in Sabouraud's dextrose agar (SDA). Antifungal activity of the Aloe vera gel and Triphala was tested by the disc diffusion method, and minimum inhibitory concentration was determined by the broth microdilution method. **Results:** The mean value for zone of inhibition for Aloe vera was 3.35 ± 0.59 mm and 1.06 ± 0.41 mm at 100% and 50%, respectively. For Triphala, it was 4.19 ± 0.57 mm and 1.79 ± 0.43 mm at 100% and 50%, respectively (P value < 0.001). Zone of inhibition of 100% Triphala showed higher value than that of Aloe vera at the same concentration ($P = 0.004$). Similar results were obtained at 50% concentration of Triphala and Aloe vera ($P = 0.004$). Minimum inhibitory concentration of Aloe vera gel and Triphala against *C. albicans* was 25% and 12.5%, respectively. **Conclusion:** Aloe vera gel and Triphala both showed antifungal property at higher concentrations and can be used as a promising adjunct for antifungal agents.

Keywords: Antifungal, culture, natural

INTRODUCTION

Candida is a genus of yeasts and is the most common cause of oral fungal infection. Most of the species of *Candida* are harmless commensals or endosymbionts of hosts including humans; however, when there is compromised immune system or when mucosal barriers are disrupted, they can invade and cause disease. *Candida albicans* is the most commonly isolated species and is responsible for oral thrush, pseudomembranous candidiasis.^[1]

The cost, side effects, and increased resistance to commercially available antifungal drugs had led the researchers to explore the natural herbal remedies against fungal infections.^[2,3] Traditionally used medicinal plants are rich source of antimicrobial agents and are readily available in rural areas. They are relatively cheaper than modern medicine. Secondary metabolite products, produced by medicinal plants, are an important source of many pharmaceutical drugs.^[1]

Aloe vera is a cactus like plant that belongs to the *Liliacea* family. Aloe vera gel is a mucilaginous colorless gel, secreted by the parenchymatous cells of fresh Aloe vera leaves. It contains 98–99% water and 1–2% active compounds such as aloin, aloe-emodin, aloemannan, flavonoids, aloesin, sterols, amino acids, and vitamins.^[4] Triphala means three fruits, is an Indian ayurvedic formulation comprising *Terminali chebula*, *T. Belerica*, and *Phyllantus embelica* in 1:1:1 proportion.^[5,6] Its phytochemical constituents are tannin, gallic acid, ellagic acid, phenol, and glycosides. Both Aloe vera and Triphala have antibacterial, antioxidant, antifungal, anti-inflammatory, and immunomodulatory properties.^[5-7] The effects of Aloe vera

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and Triphala extracts on oral *Candida* infection have been studied by very few researchers in India.^[8] The aim of the study was to determine the antifungal and inhibitory activities of various concentrations of Aloe vera gel and Triphala against *C. albicans*.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, New Horizon Dental College and Research Institute, Bilaspur. Ethical clearance was taken from the institutional ethical committee. Informed consent was taken from all the patients. *Candida* swab was taken from the patients suffering from pseudomembranous candidiasis using denture stomatitis and transferred to Sabouraud's broth [Figure 1]. All *Candida* isolates were confirmed by germ-tube test and spore germination test. This isolated *Candida* sample was incubated in Sabouraud's dextrose agar SDA (HIMEDIA laboratories, Mumbai) at 4°C.

Preparation of the Aloe vera extract

Thick epidermis of fresh Aloe vera leaf was removed and the solid mucilaginous gel was collected in a sterile container [Figure 2]. Ten millimeter of the gel was mixed in 100 ml of 1% dimethyl sulfoxide (DMSO) and kept at 4°C.^[3] DMSO ($\leq 1\%$) has insignificant effect on the growth of fungi and was therefore used as a solvent.^[9]

Preparation of the Triphala extract

One hundred gram of Triphala churna (Dabur Triphala churna) was mixed in 100 ml of water to make an aqueous solution [Figure 3]. Then this solution was boiled for 45 min. Later, it was cooled and filtered. This filtrate was used to check its antifungal effect. Antifungal property of Aloe vera gel and Triphala was detected using disc diffusion method.

Activation of *Candida albicans*

Loopful fungal spores were streaked on potato dextrose agar (PDA) plate and incubated at 37°C for 24 h prior to test. All plates ($n = 10$) were maintained at 4°C for further use.

Disc diffusion method

Eight millimeter wells were bored in each plate ($n = 10$ for *Candida*) having activated *C. albicans*, with an 8 mm borer in seeded agar in which 100%, 50%, 25%, and 12.5% of Aloe vera gel extract was poured. Similarly, for Triphala, 8 mm wells were bored in different plate ($n = 10$ for Triphala) having activated *C. albicans*, with 8 mm borer in seeded agar in which 100%, 50%, 25%, 12.5% of Triphala extract was poured. After it normalized to room temperature, plates were incubated at 37°C for 24 hours. The zone of inhibition was measured and recorded [Figure 4].

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was done using the microbroth dilution method. The highest dilution that yielded no single fungal colony was taken as the MIC. Aloe vera extracts were mixed with 1% DMSO to obtain various



Figure 1: *Candida* growth in Sabouraud's broth



Figure 2: Extraction of Aloe vera gel from fresh leaves in sterile plate



Figure 3: Triphala churna

concentrations of the stock i.e. 100%, 50%, 25%, 12.5%, and 6.25%. Similarly, various concentration of Triphala were obtained by mixing extract with distilled water. Equal volume of the various concentration of each extract and were mixed

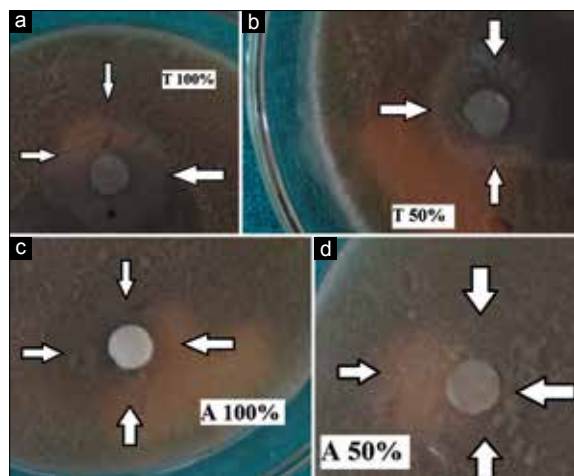


Figure 4: (a) Zone of inhibition at 100% concentration of Triphala. (b) Zone of inhibition at 50% concentration of Triphala. (c) Zone of inhibition at 100% concentration of Aloe vera. (d) Zone of inhibition at 50% concentration of Aloe vera

in micro-tubes (Sabouraud dextrose broth) to make up 0.5 ml of solution. The tubes were incubated at 37°C for 24 hours. Later, test dilution was subcultured on SDA and incubated for 24 h to check for fungal growth [Figure 5].

Statistical analysis

The data were compiled in MS Office Excel. Statistical analysis was done using SPSS version 21 software package (SPSS Statistics for Windows, Version 21.0. Chicago: SPSS Inc.). Analysis of variance (ANOVA) test was performed for intra and intergroup comparisons. *Post hoc* test (Tukey HSD) was also performed to compare different concentrations of Aloe vera, Triphala, and both. Results were measured as mean ± SD. *P* value less than <0.05 was considered to be statistically significance and <0.001 as highly significant.

RESULT

The *Candida* samples collected were analyzed by Gram staining, which showed presence of clusters of pseudohyphae suggesting *C. albicans* species.^[10] The mean value for zone of inhibition for Aloe vera was 3.35 ± 0.59 mm and 1.06 ± 0.41 mm at 100% and 50%, respectively. For Triphala, it was 4.19 ± 0.57 mm and 1.79 ± 0.43 mm at 100% and 50%, respectively [Table 1]. Both Triphala and Aloe vera gel showed antifungal property at higher concentrations (100% and 50% concentration, *P* value < 0.001).

There was highly significant difference observed between 100% and 50% Aloe vera (*P* < 0.001, *t* = 19.17). Similar results were observed for 100% and 50% Triphala (*P* < 0.001, *t* = 31.58) [Table 2]. When ANOVA was performed, degree of freedom between Aloe vera and Triphala was 3 and within groups (100% v/s 50%) it was 36 [Table 3]. On *Post hoc* test, zone of inhibition of 100% Triphala showed higher value than Aloe vera at the same concentration (*P* = 0.004). Similar results were obtained at 50% concentration of Triphala and

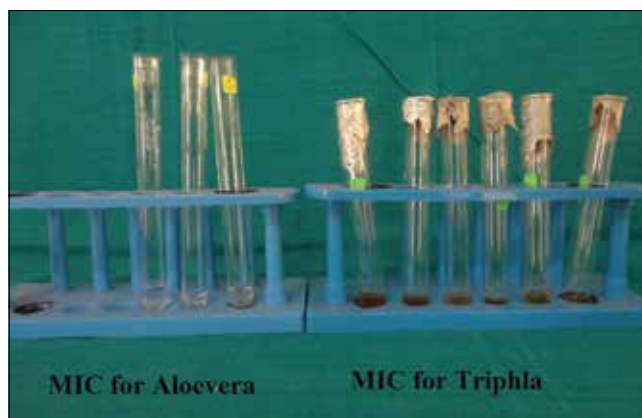


Figure 5: Minimum inhibitory concentration for Triphala and Aloe vera

Table 1: Mean value of zone of inhibition of Aloe vera and Triphala at different concentration

Concentration	N	Minimum (mm)	Maximum (mm)	Mean	Std. Deviation
Aloe Vera (100%)	10	2.00	4.10	3.3500	0.59114
Aloe Vera (50%)	10	0.40	1.70	1.0600	0.41952
Triphala (100%)	10	3.50	5.20	4.1900	0.57048
Triphala (50%)	10	1.20	2.50	1.7900	0.43063

Table 2: Student's t-test to show comparison within the group

Comparison	't' value	P
100% vs 50% Triphala	31.58	<0.001**
100% vs 50% Aloe vera	19.170	<0.001**

P<0.001; Highly significant

Table 3: ANOVA test showing degree of freedom between the groups and within the group

	Sum of squares	df	Mean square	F	Sig.
Between Groups (Aloe vera and Triphala)	61.183	3	20.394	78.717	0.000
Within Groups (100% vs 50%)	9.327	36	0.259		

Aloe vera (*P* = 0.000) [Table 4]. At lower concentration (25% and 12.5%), there was no effect against *Candida*. MIC of Aloe vera gel and Triphala against *C. albicans* was 25% and 12.5%, respectively.

DISCUSSION

The emergence of multidrug resistant (MDR) bacteria and fungi and high cost of pharmaceutical drugs have necessitated the development of alternative drugs from natural and other sources. Medicinal plants, which are being used since ancient times in Ayurveda, could be one of those alternatives as they are cheap, safe, and are effective against many microbes.^[11]

Table 4: Post hoc Test (Tukey HSD) showing comparison within groups

Groups	Mean difference	Std. error	Significance	95% Confidence interval	
				Lower limit	Upper limit
Aloe vera (100%)					
Aloe vera (50%)	2.29000	0.22763	0.000	1.6769	2.9031
Triphala (100%)	-0.84000	0.22763	0.004	-1.4531	-0.2269
Triphala (50%)	1.56000	0.22763	0.000	0.9469	2.1731

Many researchers have checked antibacterial effect of Aloe vera and Triphala.^[3,4,7,12] Very few studies have been conducted to check the antifungal effect of these plants against oral candidial infection.^[12-18] The present study was conducted to determine the antifungal and inhibitory activities of various concentrations of Aloe vera gel and Triphala against *C. albicans*. In the present study, both Triphala and Aloe vera showed anticandidal property at 100% and 50% concentrations. No significant changes were seen at lower concentration (25% and 12.5%).

Sheta *et al.* used 0.5% DMSO and distilled water as solvent and mixed Triphala (suspension) to get a final concentration of 100 mg/ml. They found that zone of inhibition for Triphala against *Candida* was 12 mm when water was used as solvent and 20 mm when DMSO was used as solvent.^[12] Authors have also checked antifungal property for DMSO and found that it has no antifungal effect. They did not boil water suspension, which could be the cause of better results than our study. Randhawa suggested that DMSO increases the permeability of cell by binding with cell membrane, thus causing synergistic effect with anticandidal drugs.^[9] In the presented case, water was used as a solvent for Triphala. El-Mekkaway *et al.* found that *T. chebula* may inhibit *Candida* species (including clotrimazole-resistant *C. albicans*).^[11] Malhotra *et al.* in their *in vitro* study found that colony forming units (CFUs) of *C. albicans* dropped to zero after 10 min by use of 6% Triphala mouthwash.^[13]

Khaing found that ethanolic extract of Aloe vera has no anticandidal effect.^[14] A similar study by Kaur *et al.* showed Aloe vera to be effective against *C. albicans*.^[15] They suggested that a positive result in their study could be because of change in procedure of gel extraction, i.e., they extracted gel at room temperature whereas a prior study extracted gel at 90°C. Hence, the active component might have been lost in the ethanolic extract if extraction was done at a higher temperature.

A study by Stanley *et al.* found that zone of inhibition for Aloe vera against *C. albicans* was 4 mm and MIC was 50%, which was similar to the present study.^[16] Shireen *et al.* found that antifungal property of Aloe vera is dose dependent. They took Aloe vera juice (1000, 500, 250, 100 µg) from fresh leaves and mixed it with 10 ml of ethanol. Zone of inhibition was 14 mm against *C. albicans* at 1000 µg concentration, which was comparable to amphotericin B.^[17] Chainani *et al.* used ethanolic extract of Triphala and found that it showed anticandidal effect at concentration 5% and above.^[18] Triphala showed better results when compared with Aloe vera, with

greater zone of inhibition in both 100% and 50% concentration. Moreover, Triphala inhibits *Candida* growth at lower concentration (12.5%) when compared to Aloe vera (50%).

The antimicrobial effect of Aloe vera is attributed to the natural component called anthraquinones (emodin, aloetic acid, aloin, anthracene, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamic acid, isobarbaloin, and resistannol).^[17,19] The purified aloe protein of 14 kDa from Aloe vera leaf gel exhibited a potent antifungal activity against *C. albicans*.^[19] Antifungal effect of Triphala is due to its two component [*T. Chebula* (Harde), *T. Belerica* (Baheda)].^[12]

We have found that both Triphala and Aloe vera have shown antifungal activity against *C. albicans*. However, the mechanism of action of these herbal agents on microbes is yet to be understood. Furthermore, more studies are required on various fungal loads and various concentrations of both Triphala and Aloe vera to check its potency against various *Candida* species.

CONCLUSION

Increase in fungal pathogens, limited therapeutic options, side effects of therapeutic drug, and emergence of MDR together put a burden on patients with compromised immunity. *C. albicans* is the most common opportunistic infection of oral cavity, frequently isolated from tissue samples of affected patients. Traditional medicines have used plant products since ages. Because of their ease of availability and less toxic effect, efforts have been made for developing therapeutic options using molecules from plant sources. This study is also a similar effort to check the antifungal effects of Triphala and Aloe vera. In this study, Aloe vera and Triphala were found to show antifungal activity.

Limitations

The present study was conducted in *in vitro* and involved a small sample size. In future, more trials should be carried out on larger population (*in vivo*) to prove the antifungal efficacy of Aloe vera and Triphala against *C. albicans* to characterize the mechanism of action for further translation into clinical use and overcoming the challenges of fungal therapeutics.

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Conflicts of interest

There are no conflicts of interest.

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