

## IN VITRO ANTIFUNGAL ACTIVITY OF ALOE VERA AND CYMBOPOGON CITRATUS AGAINST CANDIDA ALBICANS

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### ABSTRACT

**Background and Objectives:** Efficacy of antifungal agents is limited by incomplete eradication and toxicity, demonstrating need to investigate better agents. The present study was designed to determine the antifungal activity of *Aloe vera* and *Cymbopogon citratus* extracts against *Candida albicans* and to compare their antifungal effect with that of terbinafine and with each other.

**Methods:** Clinically diagnosed and confirmed cases of cutaneous candidiasis were selected. Specimens of skin scraping were taken from each individual and were inoculated on Sabouraud dextrose agar for seven days. Yeast was sub cultured on three Petri dishes to assess the antifungal activity of extracts of *Aloe vera*, *Cymbopogon citratus* and terbinafine by disc diffusion method.

**Results:** Mean zone of inhibition was  $14.6 \pm 1.59$  mm (100%) with terbinafine,  $9.6 \pm 0.43$  mm (66%) with *Aloe vera* and  $6.87 \pm 0.8$  mm (47%) with *Cymbopogon citratus*. Statistically the difference in zone of inhibition of terbinafine, *Aloe vera* and *Cymbopogon citratus* was highly significant ( $p$ -value = 0.000).

**Conclusion:** Taking antifungal activity of terbinafine as 100%, it was 66% with *Aloe vera* and 47% with *Cymbopogon citratus* which are considered intermediate.

**Keywords:** *Aloe vera*, *Cymbopogon citratus*, terbinafine, *Candida albicans*, antifungal activity.

### INTRODUCTION

*Candida* is the most common candidae species of pathogenic fungi. There are 150 – 200 different species of which *Candida albicans* is most common. Candidiasis is manifested in different forms, varying from local to systemic infection.<sup>1</sup> It can also cause a life-threatening infection of the blood called disseminated candidiasis. As they are commensal of the digestive and urogenital tracts, so candidiasis can begin endogenously. Exogenous sources of candidiasis include transfer of *Candida* from patient to patient or from healthcare staff to patient. *Candida* species are notorious opportunistic pathogens, found in all ages especially in immunocompromized, debilitated patients, patients on broad-spectrum antibiotics, steroids, cytotoxic therapy and in diabetics.<sup>2</sup>

Antifungal drugs available in the market, including terbinafine, are encountered with emerging resistance.<sup>3</sup> Terbinafine is used as a topical and systemic agent for the treatment of mycoses. Its topical use is safer as compared to oral administration because adverse effects of topical application are seen only at the site of application which includes local irritation, burning sensation, and erythema.<sup>4</sup> Terbinafine is metabolised by the enzyme CYP2D6 and it may interfere with

the metabolism of other drugs using the same enzyme.<sup>5</sup> It is not recommended for breast feeding mothers.<sup>6</sup>

Plant-based drugs have attained great attention because of few side effects, economy and easy availability. There are many plants with remarkable medicinal properties. *Aloe vera* and *Cymbopogon citratus* (lemon grass) are examples of such plants with multiple properties including antifungal activity.<sup>7</sup> In this study antifungal activity of ethanolic extract of *Aloe vera* and methanolic extract of *Cymbopogon citratus* was studied against *Candida albicans* in comparison with terbinafine.

### MATERIAL AND METHODS

This study was conducted in Pharmacology Department, Post Graduate Medical Institute, Lahore in collaboration with Department of Dermatology and Pathology, King Edward Medical University, Lahore.

### Plant Collection and Preparation of Extracts:

The plants of *Aloe vera* and *Cymbopogon citratus* were taken from plant nursery in WAPDA Employees Housing Society, Lahore and were identified and verified by the Department of Botany, The University of

the Punjab Lahore. Whole leaves of identified *Aloe vera* and *Cymbopogon citratus* were thoroughly washed, dried under shade at 35 – 40°C, and cut into small pieces. Extracts of *Aloe vera* and *Cymbopogon citratus* were prepared separately with ethanol and methanol (1:10 w/v) respectively in PCSIR laboratories, Lahore. The resultant extracts were preserved in airtight glass bottles stored at 4°C<sup>8</sup> and were dissolved in distilled water before use.

**Terbinafine Solution:** Terbinafine hydrochloride (pharmaceutical grade) was purchased from Novartis in powder form and was dissolved in distilled water to obtain a concentration 0.25 µg/ml.<sup>9</sup>

**Disc Preparation:** The discs, 5.0 mm in diameter and 1mm in thickness, were prepared from Whatmann filter paper No.1. These discs, labeled as A (*Aloe vera*), C (*Cymbopogon citratus*) and T (terbinafine) were sterilized by autoclaving at 121°C. After sterilization the discs labeled as “A” were impregnated with 20 µl (2µg) of *Aloe vera* (100 µg/ml), the discs labeled as “C” were impregnated with 20 µl (2 µg) of *Cymbopogon citratus* (100 µg/ml), and the discs labeled as “T” were impregnated with 20 µl (0.005 µg) of terbinafine (0.25 µg/ml).<sup>10</sup>

## METHODOLOGY

Skin scrapings from clinically diagnosed cases of candidiasis were collected from September- December, 2011. After microscopy identified specimens of *Candida albicans* from five patients were further confirmed by growing them on Sabouraud dextrose agar (SDA) medium in aerobic environment at room temperature ranging between 35 – 37°C (during research period) for seven days. Each pathogen was sub cultured on three agar plates. The already prepared discs were placed with equal distance on these plates and incubated aerobically at 37°C for three days.<sup>11</sup> The inhibition zone around each disc was measured in millimeters. The readings from three plates for each sample were taken and the mean was calculated.

## Statistical Analysis

The data was analyzed by SPSS, version 20. Zone of inhibition was expressed as mean ± standard deviation. One way ANOVA was applied to compare antifungal activity of three treatments. Post hoc Tukey’s test was applied to observe difference among treatments. A p-value of ≤ 0.05 was considered significant. Antifungal activity of both extracts against *Candida albicans* was also expressed in percentage, taking antifungal activity of terbinafine as 100%.

## RESULTS

Fig 1 shows zones of inhibition by terbinafine, *Aloe vera* and *Cymbopogon citratus* on growth of *Candida albicans* by disc diffusion method. Mean zone of inhibition was 14.6 ± 1.59 mm with terbinafine, 9.6 ± 0.43

mm with *Aloe vera* and 6.87 ± 0.8 mm with *Cymbopogon citratus*.

**Table 1:** Multiple comparison (Tukey’s test) of antifungal activities of terbinafine, *Aloe vera* and *Cymbopogon citratus* against *Candida albicans* run in triplicate (n=5).

	(I) Groups	(J) Groups	p-value
<i>Candida albicans</i>	Terbinafine (control)	<i>Aloe vera</i>	0.000
		<i>Cymbopogon citratus</i>	0.000
	<i>Aloe vera</i>	<i>Cymbopogon citratus</i>	0.004

Statistically the difference, in zone of inhibition of terbinafine, *Aloe vera* and *Cymbopogon citratus* was highly significant (p-value = 0.000). Effect of *Aloe vera* and *Cymbopogon citratus* was significantly less as compared to that of terbinafine and effect of *Aloe vera* was significantly high as compared to that of *Cymbopogon citratus* as shown in table 1. Taking antifungal activity of terbinafine as 100%, it was 66% with *Aloe vera* and 47% with *Cymbopogon citratus* which are considered intermediate.

## DISCUSSION

*Candida albicans* is getting resistant to many antifungal drugs including Terbinafine.<sup>12</sup> Thus this study was planned to evaluate the in-vitro antifungal activity of *Aloe vera*, *Cymbopogon citratus* and terbinafine on clinical isolates of *Candida albicans* species. According to this study, the mean zone of inhibition of *Candida albicans* growth was 9.6 ± 0.43 mm with disc impregnated with ethanol extract of *Aloe vera*. Another study demonstrates 13 mm ± 0.0 mm zone of inhibition by well diffusion method using ethanol extract of *Aloe vera*. This difference may be due to use of higher concentration of higher amount of *Aloe vera* extract i.e. 2.5 mg in each well.<sup>13</sup> A still higher value i.e., 30 mm zone of inhibition around the discs of *Aloe vera* was observed on *Candida albicans* isolates by Alemdar and Agaoglu,<sup>14</sup> and it may be due to the use of pure culture of *Candida albicans* strain (ATCC 60192) from culture collection and discs containing the 30 µl of pure *Aloe vera* juice in their study as compared to 20 µl of ethanol extract of *Aloe vera* in present study. The antifungal activity of ethanolic extract of *Aloe vera* is due to the presence of tannins and phenolic acid polyphenols, quercetin, flavones, flavonols, alkaloids, terpenoids, lectins, polypeptides, and complex mixtures.<sup>13</sup>

It was observed that extract of *Cymbopogon citratus* showed mean zone of inhibition of 6.87 ± 0.8 mm against *Candida albicans*. This observation complements the study of Ewansiha *et al*<sup>15</sup> in which, the mean zone of inhibition with methanol extract of *Cymbopo-*

*gon citratus* against *Candida albicans* was  $7.66 \pm 0.58$  mm. A slightly higher value of zone of inhibition i.e. 8 mm with *Cymbopogon citratus* against *Candida albicans* was observed in the study conducted by Hamza *et al.*<sup>16</sup> The difference in the observation may be due to the fact that *Candida albicans* were obtained from public health laboratories, which were standard laboratory isolates rather than clinical isolates. There was also a difference of growth medium for the fungal pathogen; potato dextrose agar instead of Sabouraud dextrose agar was used. Another study, conducted by Okigbo and Mmeko,<sup>17</sup> made the observation of a still higher value of mean zone of inhibition ( $9 \pm 1.73$ ) of *Candida albicans* around disc of *Cymbopogon citratus*. This difference in the observation may be due to the use of a different solvent for extraction. The solvent used was ethanol while in our study methanol was used for the preparation of extract. Thus the use of a dissimilar solvent might have the effect on the extraction of different phytochemical substances from *Cymbopogon citratus* leaves. The active principle proposed to be responsible for antifungal activity of *Cymbopogon citratus* is Geraniol, Neral, and Limonene.<sup>18</sup> *Cymbopogon citratus* has also shown to inhibit adhesions of *Candida albicans* in human buccal epithelial cells *in vitro*.<sup>19</sup>

This work has indicated that ethanol extract of *Aloe vera* and methanol extract of *Cymbopogon citratus* possess intermediate antifungal activity against *Candida albicans*. Both the herbs have low antifungal activity as compared to terbinafine. Had the combination of both agents been used, better results would have been obtained.

We can **conclude** from this study that both *Aloe vera* and *Cymbopogon citratus* can be utilized in future for the treatment of superficial candidiasis.

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#### Authors' Contribution

RK: Gave concept and design of the study. She did data collection and statistical analysis. SK: Did literature search, writing and editing of manuscript. SC: Did research supervision, review and final approval of manuscript

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