

The effect of *Aloe vera* ethanol extract on the growth inhibition of *Candida albicans*

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ABSTRACT

Aim *Candida albicans* can cause two major types of infections: superficial infections (such as oral or vaginal candidiasis) as well as life-threatening systemic infections, and *Aloe vera* extract is one of the potentially useful therapeutic options. The aim of this study was to determine antifungal properties of *Aloe vera* ethanol extract on vulvovaginal candidiasis caused by *Candida albicans*.

Methods *Aloe vera* ethanol extract was prepared by the maceration method with 70% ethanol and dissolved in DMSO into multiple concentrations (6.25%, 12.5%, 25%, and 50%). *Candida albicans* was cultured in Sabouraud dextrose agar for 72 hours and disc diffusion method was used to evaluate the inhibitory effect of each concentration in comparison with fluconazole. Zones of inhibition at 72 hours were measured and documented, then analysed to get the mean inhibition zone (MIZ).

Results After 72 hours, all concentrations of *Aloe vera* ethanol extract showed inhibition effect against *C. albicans* with mean inhibition zones of each concentration, 12.450±0.208 mm (6.25%), 13.975±0.457 mm (12.5%), 15.650±0.420 mm (25%), and 17.225±0.512 mm (50%), respectively. Fluconazole revealed comparable antifungal effect with MIZ of 11.025±0.478 mm. One-way ANOVA test showed a significant effect of *Aloe vera* ethanol extract on inhibition zone of *Candida albicans* growth ($p < 0.005$).

Conclusion *Aloe vera* ethanol extract possesses concentration dependent activity against *Candida albicans* that is comparable with standard antifungal agents.

Key words: herbal, therapy, yeast infections

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INTRODUCTION

Candida species is one of the commensal microorganisms in humans that can be found on the skin, gastrointestinal, genitourinary tracts and in the oral and conjunctival space (1). There are several *Candida* species, such as *Candida* (*C.*) *albicans*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, and *C. glabrata* (1,2).

C. albicans is a polymorphic fungus that can grow either as ovoid-shaped budding yeast, as elongated ellipsoid cells with constrictions at the septa (pseudohyphae) or as parallel-walled true hyphae (3). *C. albicans* can cause two major types of infections in humans: superficial infections (such as oral or vaginal candidiasis) and life-threatening systemic infections (4). It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC), and 40–50% experience at least one additional episode of infection (5). A small percentage of women (5–8%) suffer from at least four recurrent VVC per year.

Predisposing factors for VVC include diabetes mellitus, use of antibiotics, oral contraception, pregnancy and hormone therapy (6). The clinical signs of VVC are oedema and erythema of the vulva and the vagina accompanied by an abnormal vaginal discharge that may be watery, cheese-like, or minimal. The vaginal discharge typically resembles cottage cheese (7).

Recently, there has been an increasing challenge for patients with refractory vulvovaginal candidiasis caused by azole-resistant *Candida* species. Fluconazole resistant *C. albicans* is a growing and perplexing problem following years of indiscriminate drug prescription and unnecessary drug exposure and for which there are few therapeutic alternatives. Regrettably, although the azole class of drugs has expanded, new classes of antifungal drugs have not been newly registered, so limited treatment options exist for patients with azole resistant *Candida* vaginitis (8). Potential alternative therapies include the use of new active principles, as natural products, that have been active *in vitro*. Among the natural products, plants contain diverse components that are important sources of biologically active molecules (1).

Many plants have been utilized for their medicinal properties worldwide. *Aloe vera* species has

been used in folk medicine for over 2000 years and has remained an important component in the traditional medicine of many countries. *Aloe vera* belongs to the *Liliaceae* family. It is reported that *Aloe vera* contains over 75 nutrients and 200 active compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids, and other different potentially active compounds including simple/complex polysaccharides, phenolic compounds, and organic acids (9). Based on *in vitro* and animal studies, which used total leaf extract, *Aloe vera* exhibits anti-inflammatory, anti-arthritic, antibacterial, and hypoglycemic properties (10). Several studies have proven the antifungal properties of *Aloe vera* extract on the inhibition of *Candida albicans* growth (11). However, we have not found any study in the usage of *Aloe vera* on human vulvovaginal candidiasis.

The aim of this study was to determine the antifungal properties of *Aloe vera* ethanol extract on vulvovaginal candidiasis species including *Candida albicans*.

MATERIAL AND METHODS

Study design and sample selection

This laboratory-controlled prospective study was conducted at the Microbiology Faculty of Universitas Sumatera Utara in the period June to October 2018. *Aloe vera* extract was obtained from Pharmacology Laboratory, Pharmacology Faculty of Universitas Sumatera Utara. The inclusion criteria were the number of 0.5 McFarland standard, which consists of 1.5×10^8 colony/mL of *Candida albicans* on Sabouraud dextrose Agar (SDA). The exclusion criteria was the culture contaminated with bacteria.

The protocol of this study was approved by the Health Research Ethical Committee, Universitas Sumatra Utara/H. Adam Malik General Hospital, Medan, Indonesia.

Methods

Preparation of extract. Dried *Aloe vera* in the powder form as much as 7 g, extracted by using maceration method with 70% ethanol. The maceration process was carried out for 3 days with occasional stirring. Maceration solution was then filtered to obtain filtrate. Maceration filtrate was

concentrated to obtain as much as 1.5 gr extract. The result of the marinade extract was 21.42% *Aloe vera* extract. This thick extract was left alone at room temperature until all the ethanol evaporated. Then the extract was stored in a sealed bottle before the examination.

In vitro test. The tested fungal isolates were *C. albicans* obtained from vulvovaginal candidiasis patient at Microbiology Laboratory of Sumatera Utara Universitas. The selected microorganism was identified and confirmed by conventional and biochemical test. *Candida albicans* streaked on SDA plates using sterile swabs, previously suspended in sterile distilled water (for 15 minutes) and adjusted to 1×10^6 colony forming units (CFU/mL) (0.5 McFarland standard) using a nephelometer.

Antifungal activity of *Aloe vera* extracts was evaluated using disc diffusion method. The inoculum size of each clinical isolate was standardized matching a turbidity equivalent to a 0.5 McFarland standard. A total of 24 cultures were divided into 4 *Candida* species in 6 test groups, as followed: group I (positive control by using fluconazole, 50 mg/mL), group II (negative control by using 1% DMSO solution), group III (using 50% *Aloe vera* extract in 70% ethyl ethanol, diluted in 1% DMSO), Group IV (using 25% *Aloe vera* extract in 70% ethyl ethanol, diluted in 1% DMSO), group V (using 12.50% *Aloe vera* extract in 70% ethyl ethanol, diluted in 1% DMSO), and group VI (using 6.25% *Aloe vera* extract in 70% ethyl ethanol, diluted in 1% DMSO). The swab was drawn over the entire surface of already prepared plates of SDA to get a uniform distribution of bacteria. The SDA plates were then kept lid side up in an incubator at 25 °C for 72 hours. The plates were checked daily for spillage and growth of other organisms. Measurement of the inhibition zone was done on the third day when the margin of inhibition was clearly visible. The zones of complete inhibition were measured using a Vernier caliper in millimetres by gross visual inspection.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA test. The $p < 0.05$ was considered statistically significant.

RESULTS

After 72 hours, all concentrations of *Aloe vera* ethanol extract showed inhibition activity on *Candida albicans*. Minimum concentration of the extract to have antifungal activity was 6.25% with the mean inhibition zone of 12.450 ± 0.208 mm, followed by 12.5% with the mean inhibition zone of 13.975 ± 0.457 mm, 25% with the mean inhibition zone of 15.650 ± 0.420 mm, and 50% with the mean inhibition zone of 17.225 ± 0.512 mm. These results are comparable with or even more superior than fluconazole (positive control), which showed the inhibition zone of 11.025 ± 0.478 mm.

A significant effect of *Aloe vera* ethanol extract on the inhibition zone of the growth of *Candida albicans* was found ($p < 0.005$). Each concentration showed distinct significant results in the inhibition of the growth of *Candida albicans* (Table 1, Figure 1).

Table 1. Distribution of the inhibition zone of *Candida albicans* by *Aloe vera* ethanol extract

Groups	Diameter of inhibition zone (mm)		
	Mean±SD	Minimum	Maximum
Positive control	11.025±0.478	10.50	11.60
Negative control	0.0000	0.00	0.00
50% <i>Aloe vera</i> ethanol extract	17.225±0.512	16.70	17.80
25% <i>Aloe vera</i> ethanol extract	15.650±0.420	15.20	16.10
12.5% <i>Aloe vera</i> ethanol extract	13.975±0.457	13.50	14.50
6.25% <i>Aloe vera</i> ethanol extract	12.450±0.208	12.20	12.70

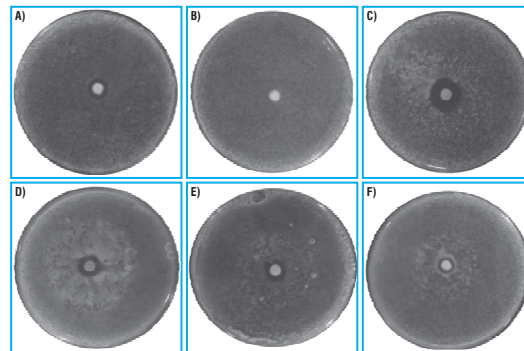


Figure 1. Inhibition zone of *Candida albicans* on Sabouraud dextrose agar for four different *Aloe vera* ethanol extract concentrations (disc in the center): A) positive control showed a clear inhibition zone around disc diffusion of fluconazole, B) negative control showed no inhibition zone around disc diffusion, C) 50% concentration showed a mean inhibition zone of 17.225 ± 0.512 mm, D) 25% concentration showed the mean inhibition zone of 15.650 ± 0.420 mm, E) 12.5% concentration showed the mean inhibition zone of 13.975 ± 0.457 mm, F) 6.25% concentration showed the mean inhibition zone of 12.450 ± 0.208 mm

DISCUSSION

In the present investigation, *in vitro* anti-fungal activity of the ethanol extracts of *Aloe vera* on

pathogenic VVC species, *Candida albicans*, was quantitatively evaluated on the basis of the zone of inhibition. All four concentrations of *Aloe vera* ethanol extracts exhibited a varying degree of inhibitory effect against selected fungal pathogens. The highest concentration of *Aloe vera* ethanol extracts performed the maximum zone of inhibition.

In Mpila et al. study, it was stated that antimicrobial activity of Mayana leaves (*Coleus atropurpureus [L] Benth*) ethanol extract to the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* by active ingredients could be grouped into four categories as weak, moderate, strong and very strong (inhibition zone <6 mm, 6-10 mm, 11-20 mm and 20-30 mm, respectively) (12). The results of our study have shown *Aloe vera* ethanol extracts with the concentration of 6.25%, 12.5%, 25%, and 50% strong growth inhibition on *Candida albicans*.

The study by Kurniawan et al. reported that ethanol extract of *Aloe vera* contained flavonoid, alkaloids, tannins, saponins and steroids (10,13). These compounds were considered to play a role in inhibiting the growth of *Candida albicans* (10). Flavonoids can cause coagulation or clumping of proteins. Clotting proteins undergo denaturation so they cannot function anymore (14). Tannins can interfere with the function of the cytoplasmic membrane. At low concentrations it can damage the cytoplasmic membrane causing leakage of important metabolites that activate the enzyme system, whereas at high concentrations it can damage the cytoplasmic membrane and precipitate cell proteins. Saponins can reduce surface tension resulting in increased permeability or cell leakage and cause intracellular compounds to come out. Steroids can inhibit protein synthesis because they accumulate and cause changes in cell constituent components (14,15). Terpenoids exhibited excellent activity against *C. albicans* yeast and hyphal form growth. Thus, terpenoids may be useful in the near future not only as an antifungal chemotherapeutic agent but also to synergize effects of conventional drugs like fluconazole (15).

A study by Huslina (16) reported that the administration of *Aloe vera* leaf extract affects the length of the inhibition zone in the growth of *C. albicans*: the greater concentration of *Aloe vera* leaf extract is given, the greater the zone of growth inhibition

of *C. albicans* is formed. *Aloe vera* leaf extract with concentrations of 100%, 50% and 25% have the inhibitory capabilities that are equivalent to nystatin 0.50 mg, 0.24 mg and 0.20 mg, respectively. A study by Renisheya et al. reported anti-bacterial and antifungal activity of *Aloe vera* gel extract on pure five bacterial (*Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Proteus vulgaris*) and three fungal (*Aspergillus fumigatus*, *Candida albicans* and *Penicillium* sp.) cultures. They found that the maximum inhibition zone of 11 mm for *C. albicans* and 9 mm for *Penicillium* sp. (17). A different study by Saniasiaya et al. (10) reported antifungal effect of the Malaysian *Aloe vera* leaf extract on selected fungal species of pathogenic otomycosis species in a culture medium. For *Aspergillus niger*, a zone of inhibition for alcohol and aqueous extract was seen for all concentrations except 3.125 g/mL; there was no zone of inhibition for both alcohol and aqueous extracts of *Aloe vera* leaf for *C. albicans* (10). The preparation of the *Aloe vera* extract at high temperature may have affected the active ingredient leading to the ineffectiveness towards *C. albicans* (18).

In conclusion, the extract of *Aloe vera* has shown concentration-dependent antifungal effect on *Candida albicans*. A possible mechanism of the obtained antifungal properties is related with flavonoids, alkaloids, tannins, saponins and steroids/terpenoids, contained in *Aloe vera* extracts. Comparable inhibitory effect with fluconazole might support the application of these extracts like efficient antifungal agents. Therefore, further clinical trials are needed to confirm efficacy and safety of *Aloe vera* extracts as potential antifungal agents.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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