Original Research

Anti-fungal activity of *Aloe vera*: *In vitro* study

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ABSTRACT

Aim: The aim of this study was to investigate the anti-fungal activity of *Aloe vera* extract on *Candida albicans*. **Materials and Methods:** Extract from *A. vera* fruit was tested for anti-fungal activity via *in vitro* study at various concentrations using the disc diffusion method. **Results:** *A. vera* extract at 1000 μg/ml concentration effectively inhibited the growth of *C. albicans* (14 mm) compared with the positive control-amphotericin B (15 mm). It was found to be a dose-dependent reaction. **Conclusion:** *A. vera* displayed good anti-fungal effect on *C. albicans* and the inhibitory effect varied with concentration.

Key words: *Aloe vera*, anti-fungal, *Candida albicans*

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic fungal microorganisms is the leading cause of morbidity and mortality in immunocompromised patients in developing countries.[1] This is further worsened by the situation of multi-drug resistant strains of bacteria due to the increase in the use of antibiotics and there remains a paucity of newer group of antibiotic drugs.[2,3] Despite the existence of potent antibiotic and anti-fungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. Plants are the cheapest safer and time-tested alternative sources of antimicrobials.[4-6] In ancient times, people believed that plants had curative powers. Phytotherapy or phytomedicine has been a part of both Eastern and Western medical traditions since 2000 BC. Literature shows that the Chinese used ginseng at least 3000 years ago, Native Americans used willow bark tea to reduce fever. Each civilization that has progressed has stressed the use of medicinal plants. The recent increase in the popularity of herbals products globally may reflect the fact that a lot of people have disbelief with the current allopathic medical practice.[5] People feel that using herbal extracts caters to purity, simplicity, and safety. Most people feel that herbal medicines are safer and less toxic. Popularly used herbal supplements in the dental field are licorice, ginger, ginseng, garlic, and clove.[6] Much of the information available herbal supplements are market driven and not supported by clinical research studies. Moreover, the quality, strength, and purity of the medication depend on the time, place, and season of cultivation apart from the techniques used in processing and packing.

The practice of alternative medicine is now on the rise in developing countries due to the World Health Organization support and propagation on the scientific basis for the efficacy of many plants used in folk medicine to treat infections.[6,7]

Aloe vera is a well-known medicinal plant belonging to the Liliaceae family. It is a cactus-like plant that grows readily in hot tropical climates. The slimy gel in the A. *vera* leaf (A. *vera* gel) has traditionally been used for treatment of the digestive tract disturbances, sunburn and wounds and it has been attributed to more than 75 active agents. The gel consists of 98-99% water and the remaining 1-2% contains the active compounds, such as aloesin, aloin, aloe-emodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino

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acids, and vitamins. The levels of these compounds vary according to species, strain, and growth conditions. The pharmacological actions of A. *vera* gel as studied *in vitro* and *in vivo* include anti-inflammatory, antibacterial, antioxidant, immune-boosting and hypoglycemic properties.[8-10] In the present study, we investigated the anti-fungal activity of A. *vera* gel in order to find its potential applicability in the field of dentistry.

MATERIALS AND METHODS

Aloe vera is a stemless or very short-stemmed succulent plant growing to 60-100 cm (24-39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. A. *vera* leaves contain phytochemicals under study for a possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, other anthraquinones, such as emodin and various lectins. The leaf was cut, and the juice that drained from it was freshly collected in a plastic container. 1000, 500, 250 μg, and 100 μg were diluted in 10 ml of ethanol. The sample was kept aside for 24 h and was stirred occasionally.

Preperation of the assay

The Kirby-Bauer method was used. Crude ethanolic extract of A. *vera* was used. Standard positive control for *Candida albicans*-amphotericin B (concentration 10 μg/disc) was used. The leaf was cut, and the juice that drained from it was freshly collected in a plastic container. 1000, 500, 250 and 100 μg was diluted in 10 ml of ethanol. The sample was kept aside for 24 h and was stirred occasionally. The samples were then subject to microbial growth after 24 h.

Preparation of inoculum

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loopfull of cells from the stock cultures to test tubes of nutrient broth for fungi that were incubated at 24 h at 37°C. The assay was performed by agar disc diffusion method.

Disc diffusion method

Anti-fungal activity of the given sample was determined by disc diffusion method on Muller-Hinton agar (MHA) medium. The MHA medium is poured into the petriplate. After the medium had been solidified, the inoculums were spread on the solid plates with sterile swab moisture with the bacterial suspension. The discs were placed on MHA plate with the help of sterile forceps and different concentrations (1000, 500, 250, and 100 μg) of each sample were loaded on the discs. The plates were incubated for 24 h, at 37°C. Then the microbial growth was determined by measuring the diameter of the zone of inhibition.

RESULTS

Table 1 and Figure 1 show the anti-fungal activity of A. *vera* versus the control. The center disc represents the positive control amphotericin B. The dimethyl sulfoxide was not used here as it was to observe fungal activity. Anti-fungal activity appeared at 250 μg it was 7 mm, at 500 μg it was 9 mm and at 1000 μg it was 14 mm. The activity increased with increasing concentrations. It was as effective as the positive control amphotericin B which showed 15 mm of zone of inhibition.

DISCUSSION

Oral opportunistic fungal infections are chronic and ubiquitous in nature. They have a slow rate of progression and are often asymptomatic for large number of diseased years only when their growth is significantly large the problem arises. But usually by then the problem has reached a sufficient magnitude that it is nearly difficult to treat and eradicated it completely. With candida infection, the problem of recurrence is very common. The problem is exemplified with the increase in the growth of the resistance of the organism to the anti-fungal medication.

A majority of the Indians believe that the age-old grandmas remedies help in most occasions. A. *vera* of late is gaining popularity in the field of dental medicine in this regard. The present study shows that the gel has a potent anti-fungal activity. This is in agreement with studies done by George *et al.*[11] and Heggers *et al.*[12] The activity increases with the increase in the dose. This is also in agreement with studies done by Heggers *et al.*[12]

Table 1: Antifungal activity of *Aloe vera* gel

Microorganism	1000 μg	500 μg	250 μg	100 μg	50 μg	Amphotericin B
Candida albicans	14±0.3	9±0.2	7±0.3	—	—	15±0.3

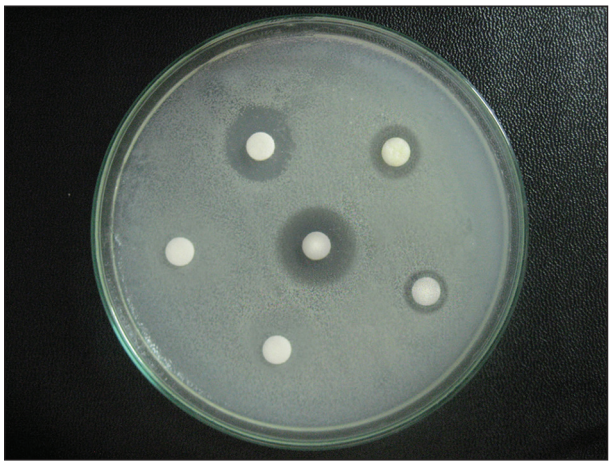

Figure 1: The anti-fungal activity of *Aloe vera*

The present study highlights the fact that A. *vera* gel proves to be effective in the case of opportunistic fungal infections especially for the immunocompromised subjects like organ transplant patients HIV subjects as this will reduce the burden of the medication. The usage of A. *vera* will also be cost effective as the cultivation is relatively easy. One can almost cultivate it in the backyard of their own homes.

Aloe vera contains 75 potentially active constituents that include vitamins, minerals, sugars, lignin, saponins, salicylic acids, amino acids, and enzymes.[13] The activity of A. *vera* is attributed to the ingredients called Glucomannan and Gibberelli. The former is a mannose-rich polysaccharide, and the latter is a growth hormone which interacts with growth-factor receptors on the fibroblast, thereby stimulating proliferation, which significantly increases collagen synthesis after application.

The anti-microbial effect of A. *vera* is attributed to the component called as plant's natural anthraquinones. Anthraquinones are naturally occurring aromatic compound that are found in plants that and are applicable in the field of medicine and the dye industry. The anthraquinones found in A. *vera* are emodin, aloetic acid, aloin, anthracene, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamic acid, isobarbaloin, and resistannol.[13] As suggested by Wynn,[14] this component plays a vital role by the way of inhibiting the cyclooxygenase pathway and reduces prostaglandin E2.

Yagi *et al*.[15] reported that A. *vera* gel contains a glycoprotein with cell proliferating-promoting activity while Davis *et al*.[16] noted that A. *vera* gel improved wound healing by increasing blood supply (angiogenesis), which increased oxygenation, as a result.

Aloe-emodin in A. *vera* makes it so that certain viruses are not able to function.[17] Therefore, A. *vera* is virucidal to Herpes simplex virus Type 1 and Type 2, Varicella zoster virus, pseudorabies virus, and influenza virus according to the research of Thomson.[18]

Aloe vera has very strong antioxidant nutrients. Glutathione peroxide activity, superoxide dismutase enzymes, a phenolic antioxidant and vitamins A, C, E were found to be present in A. *vera* gel, which may be responsible for these antioxidant effects.[19] All these factors help us to understand the useful benefits of using A. *vera* in the dental field.

Potential areas of applications in dentistry:

1. Gingivitis and applications directly at sites of periodontal surgery in addition to scaling and root planning in periodontitis;[20,21]
2. Aspirin burns;[22]
3. Angular cheilitis;[23] aphthous ulcer;[24] and in the treatment of oral lichen planus;[24-26]
4. Burning mouth syndrome;[13]
5. Patients with sore gums and teeth with ill-fitting dentures maladaptive may also benefit;[27]
6. *Aloe vera* can also be used around dental conditions to control inflammation caused by bacterial contamination;[10,28]
7. Anti-cancer benefits;[29,30]
8. Alveolar osteitis;[31]
9. Wound healing.[15,32]

Though this study is done in a preliminary stage, it gives a broader idea of the strong anti-fungal properties of A. *vera*. The concentration of the usage of A. *vera* gel according to the study was found to be 1000 μg at this level optimum activity of in terms of increased minimal inhibitory concentration levels was observed for therapeutical purposes. It is recommended that further research is done on the *in vivo* effects of A. *vera* on the oral micro-organism are done to observe the effect of the gel on the microorganisms under biologic conditions. The results presented here are phase one of the research phase, two is in process to strongly validate the recommendation of the use of A. *vera* gel as a medium for oral anti-fungal use.

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