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. 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. 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 antimiorebials. 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 herbal products globally may reflect the fact that a lot of people have disbelieve with the current allopathic medical practice. 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. 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.

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, aloemodin, aloemannan, acemannan, aloeride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino
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. 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 manannans, 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.

Preparation 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 subjected 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 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 a 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 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. and Heggers et al. The activity increases with the increase in the dose. This is also in agreement with studies done by Heggers et al.

Table 1: Antifungal activity of Aloe vera gel

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>1000 µg</th>
<th>500 µg</th>
<th>250 µg</th>
<th>100 µg</th>
<th>50 µg</th>
<th>Amphotericin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>14±0.3</td>
<td>9±0.2</td>
<td>7±0.3</td>
<td>—</td>
<td>—</td>
<td>15±0.3</td>
</tr>
</tbody>
</table>

Figure 1: The anti-fungal activity of Aloe vera
Shireen and Manipal: 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 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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