Antibacterial effect of aloe vera gel against plaque and caries bacteria: an in-vitro study

Suraj Bhaiyana,1* Abhiney Puri2, Rajat Nangia3, Nitish Bhat4

1PG Student, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh
2Prof and Head, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh
3Reader, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh
4Sr. Lecturer, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh

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Abstract
The use of antibiotics has been increasing in the medical field for the control and cure of diseases but with the increase in antibiotics there is an increasing resistance of antibiotics which has made us to think about various herbal remedies which have lesser or no side effects as compared to their counterpart in chemically manufactured products. **Aim:** To check the anti-bacterial effect of Aloe Vera gel at different concentrations against plaque and caries bacteria and to compare it with antibiotics (cefexime and ofloaxacin). **Methodology:** Plaque samples were collected early morning from inter-proximal sites of lower central incisors with sterile curettes including both supragingival and subgingival plaque. Clinical isolate were then grown in Brain-Heart Infusion broth and incubated for 24 hours at 37°C. Anti-microbial activity of the Aloe Vera Gel was tested by the disc diffusion method. **Results:** Among the various concentrations of Aloe Vera, the group with Aloe Vera extract at 100% concentration showed the best results but the antibacterial efficiency was lower as considered with cefexime and ofloaxacin. **Conclusion:** It can be concluded that Aloe Vera gel can be used as an anti-bacterial agent to prevent and treat some oral infectious diseases such as dental caries, at higher concentration.

Keywords: Aloe Vera, Plaque, Caries, Antibiotic resistance, Herbal remedies.

Introduction
In the developing world the advancement in medical field has not only led to better diagnosis but also to better treatment modalities which have led to a control over many diseases, but despite these efforts some of the diseases still continue to be a major health problem, one of which includes the oral diseases.[1]

Although a decline in the dental caries prevalence is seen in most developed countries as reported according to the World Health Organization it still continues to be a major public health problem in many developing countries[2] the other oral health problems include periodontitis, pericoronitis, bacterial and fungal infections of the oral cavity. The mainstay line of treatment for many diseases in today’s world is the antimicrobial therapy which helps to control and treat infections but recently resistance to the antibiotics which help in control the infections are being documented and the resistance has been increasing day by day. This has lead us to explore natural herbs and to use them as natural

*Correspondence
Suraj Bhaiyana
PG Student, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India
E-Mail: surajbhaiyana@yahoo.com
remedies, as they have fewer side effects, for the treatment of various oral diseases and infections.[3] Among the currently available herbal agents one of the most popular and currently receiving a lot of scientific attention is Aloe Vera.[4] It grows mainly in the dry areas of Africa, Asia, Europe, and America.[5]

AIM OF THE STUDY: The aim of the present study is to evaluate the antimicrobial activities of Aloe Vera gel against plaque and caries colonizers and compare with commonly used antibiotics

**Material methods**

Collection of microbial samples: the purpose of the study was explained to all the subjects and a written consent was obtained from all the subjects.

**Dental plaque samples:** Dental plaque samples were collected from 25 students of our college. They were evaluated and selected as per following criteria:

**Inclusion criteria for plaque sample**

Age group 18 to 22, DMFT index = 0

**Exclusion criteria for plaque samples**

Subjects with any carious lesion, oral diseases, orthodontics appliances, on antibiotic therapy for past 1 month or any other immune compromised condition, presence of any oral mucosal lesion, any systemic disease like diabetes and hypertension, subject was not on any type of anticancer therapy or any kind of radiation exposure.

Sample collection: Before the collection of plaque sample subjects were advised not to brush their teeth one night before and not to eat or drink for two hours before sample collection. Plaque samples were collected early morning from inter-proximal sites of lower central incisors with sterile curettes including both supra-gingival and sub-gingival plaque. The curette was placed buccally against the tooth surface sub-gingivally and withdrawn coronally.

**Preperation of the extract**

Fresh mature leaves of Aloe Vera were washed with fresh water then their thick outer layer (epidermis) was removed and the solid mucilaginous gel was collected in a sterile container. Then 10 ml of gel was mixed in a 100 ml of 2% Dimethyl Sulfoxide (DMSO) and kept at 4°C. DMSO was used as a solvent as it has no anti-microbial effect of its own. Antibacterial property of Aloe Vera Gel was detected using disc diffusion method.

**Disc diffusion method**

Clinical isolate were then grown in Brain-Heart Infusion broth and incubated for 24 hours at 37°C. Then a 0.1 ml of the culture was poured into sterile petri plate and allowed to solidify. Wells were bored in each plate with 8 mm borer in seeded agar in which Aloe Vera Gel extract of 100%, 50%, 25%, 12.5% was poured. After normalizing to room temperature, the plates were incubated for 24 hours at 37°C. Zone of inhibition was measured and recorded. Optical density of the culture was adjusted to 0.5 with sterile Brain – Heart Infusion broth. Antibiotics [Ciprofloxacin (30mcg), Ofloxacin (5mcg)], distilled water and Dimethyl sulfoxide were also poured in different plates against same clinical isolates and zone of inhibition was measured for comparison with Aloe Vera Gel. 2% DMSO was mixed to extracts to obtain various concentration of the stock i.e., 100%, 50%, 25%, 12.5% and an equal volume of the various concentration of each extract and Brain Heart Infusion broth were mixed in micro-tubes to make the solution of 0.5 ml. The tubes were incubated anaerobically at 37°C for 24 hours. 100μl of microbial inoculums formed on the broth (plaque and caries) were aseptically introduced by micropipette and spread by using L shaped spreader on surface of brain heart infusion agar plates.

**Statistical Analysis**

The data was compiled using the MS Office Excel and thestatistical analysis was done using SPSS version 21 software package (SPSS Statistics for Windows, Version 21.0. Chicago:SPSS Inc.). For the intra and inter-group comparisons ANOVA was performed. To compare between different concentration of Aloe Vera Student’s test was also performed and results were measured as mean±SD. A p-value less than <0.05 was considered to be statistically significant and p<0.001; highly significant.

**Results**

Among the various concentrations of Aloe Vera, the group with Aloe Vera extract at 100% concentration showed the best results (Table I) but the antibacterial efficacy was lower as considered with cefixime and ofloxacin. The efficacnacy of cefixime was highest followed by ofloxacin (Table II). The p-value obtained between the 100% and 50% was also seen to be highly significant (p<0.001) as depicted in Table III.

**Discussion**

There increasing use of antimicrobial drugs has raised a concern about increasing resistance
developed by micro-organisms against antimicrobials, which has turned us towards the remedies of the past which includes the use of natural products in the prevention and treatment of various diseases including the oral conditions. This could not only be beneficial because of fewer side effects but also due to lower prices which can boost the oral health even to low socioeconomic level in urban and rural communities.[6]

The use of plants and plant derivatives which are known to possess preventive and therapeutic effects could contribute to oral health[7] one of which is a plant from Liliaceae family[8] named as AloeVera. AloeVera derived from the Arabic word “Alloeh” meaning “shining bitter substance,” while “Vera” in Latin means “true.”[4] The Aloe Vera plant ideally grows in low rainfall areas having a tropical climate.[8] AloeVera has been known to contain 75 potentially active constituents which includes : vitamins, sugars, lignin, enzymes, minerals, salicylic acids, saponins, and amino acids.[9]

The utilization of Aloe Vera in dentistry dates back to 1982 where it was utilized to cure periodontitis.[10] Many investigations have been specified the pharmacologic activities of AloeVera including its anti-inflammatory, against bacterial, hostile to viral and antifungal properties.[11] The antimicrobial effects of AloeVera has been attributed to the plant’s anthraquinones: Aloemodin, Aloein, barbaloin, Aloetic acid, chrysophanic acid, ethereal oil, anthracine, anthranol, ester of cinnamonic acid, resistanhol and isobarbaloin.[12] Polyphenolic structures, which can repress protein union by bacterial cells have been seen in Aloein and Aloe-emodin, in this way clarifying their antimicrobial action. This trademark may likewise clarify the calming action of Aloe Vera gel.[13]

The antimicrobial impact of a dentifrice containing AloeVera has been shown in an vitro study,[14] in which AloeVera restrained the development of various oral microorganisms. Studies have demonstrated that while utilizing AloeVera in tooth pastes it acts powerfully against Lactobacillus acidophilus, Enterococcus faecalis, Candida albicans, Streptococcus mutans, Peptostreptococcus and Prevotella intermedia.

AloeVera tooth gel has known to exhibited improved antibacterial activity against S. mitis.[15] Also the saponin introduce in Aloe has a lathery and purifying activity which goes about as a frothing specialist in toothpaste.

In a randomized controlled clinical trial, toothpaste containing Aloe showed an improvement in gingival and plaque index scores.[7] Villalabos et al.[16] used a mouth rinse containing only AloeVera as the active ingredient at a higher concentration of 50% and, favouring its action without interference of other components. It was concluded by Fani and Kohanteb that Aloe Vera gel at optimum concentration could be used as an antiseptic not only for prevention of dental caries but also for periodontal diseases.[3] Irshad S et al.[17] found that Aloe Vera Gel is effective against Escherichia coli, Bacillus subtilius, Salmonella typhi, Pseudomonas, Klebsiellaepidermidis. In our study we found AloeVera gel to be effective against both gram negative bacteria and gram positive bacteria. The results of our study were comparable to that of Bashir A et al.[18] in which Aloe Vera Gel for gram negative isolates was found to be 100% active and for gram positive pathogens was found to be 75.3% active. The lowest concentration at which Aloe Vera Gel is in effective in our study is 25% and at 50% and 100% it is very effective. 100% being most effective and it is comparable to cefxime and ofloxacin with no statistical difference between both.

Currently, dentifrices containing Aloe Vera Gel are under clinical trials to control the dental plaque and gingivitis. The results of the study suggested that Aloe Vera Gel has anti-microbial activity against oral pathogens especially against S. mutans which may be attributed due to the presence of active compounds mainly Aloein and Aloe-emodin (anthroquinones) which inhibit protein synthesis by bacterial cells; thus, contributing to its antimicrobial activity.

AloeVera, in tooth pastes and dentifrices leads to inhibition of dental caries and reduction of plaque due to its unique anti-bacterial properties, though studies related to its usage in dentistry is limited. So, we tried to check its anti-microbial effectiveness against oral pathogens.
Table I: Variations of extract at different concentrations

<table>
<thead>
<tr>
<th>Aloe Vera gel</th>
<th>No of plaque samples</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Mean ± SD</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>25</td>
<td>8</td>
<td>13</td>
<td>10.48 ± 2.735</td>
<td>2.735</td>
</tr>
<tr>
<td>50%</td>
<td>25</td>
<td>4</td>
<td>10</td>
<td>6.28 ± 1.926</td>
<td>1.926</td>
</tr>
<tr>
<td>25%</td>
<td>25</td>
<td>0</td>
<td>2</td>
<td>1.22 ± 0.768</td>
<td>0.768</td>
</tr>
<tr>
<td>12.5%</td>
<td>25</td>
<td>0</td>
<td>.5</td>
<td>0.35 ± 0.120</td>
<td>0.120</td>
</tr>
</tbody>
</table>

Table II: Antibacterial efficiency of other antibiotics and control group

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No of plaque samples</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Mean ± SD</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefixime</td>
<td>25</td>
<td>10</td>
<td>15</td>
<td>12.88 ± 3.003</td>
<td>3.003</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>25</td>
<td>5</td>
<td>12</td>
<td>8.24 ± 3.479</td>
<td>3.479</td>
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<tr>
<td>Distilled water</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>Dimethyl sulphoxide</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table III: Comparision of antibacterial activity of Aloe Vera at 100% and 50% concentrations with “p value”

<table>
<thead>
<tr>
<th>Concentration of aloe vera gel%</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Comparison</th>
<th>‘t’ value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>25</td>
<td>10.48 ± 2.735</td>
<td>100% vs 50%</td>
<td>7.504</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>50%</td>
<td>25</td>
<td>6.28 ± 1.926</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Conclusion
In this study we conclude that AloeVera gel can be used as an anti-bacterial agent to prevent and treat some oral infectious diseases such as dental caries, at higher concentration but in future there is a need for further clinical to test the unique antibacterial properties of AloeVera gel against various oral pathogens.

References

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Conflict of Interest: Nil