DOI: 10.7860/JCDR/2016/21450.8890

Original Article

Dentistry Section

Antibacterial Effect of Aloe Vera Gel against Oral Pathogens: An In-vitro Study

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ABSTRACT

Introduction: Natural herbal remedies have shown promising anti-microbial property and fewer side effects compared to synthetic anti-microbial therapy. Aloe Vera is a medicinal plant used for management of various infections since ancient times as it has anti-inflammatory, anti-microbial, and immune-boosting properties.

Aim: The aim of the present study was to determine the anti-microbial and inhibitory activities of various concentration of Aloe Vera Gel (AVG) against oral pathogenic bacteria.

Materials and Methods: Subgingival calculus and aspiration of periapical abscess and periodontal abscess was done in 20 patients and the sample transferred to thioglycolate broth, which was incubated in Mutans Sanguis agar, blood agar and cultured in anaerobic gas chamber. The colonies formed were identified further by gram staining methods and biochemical fermentation tests (IMViC). Each isolated colony of identified bacteria were cultured separately in Muller-Hilton broth and incubated at 37°C for 24 hours. Anti-microbial activity of the AVG was tested by the disc diffusion method and minimum inhibitory concentration was determined by broth micro-dilution method.

Result: Various staining and biochemical tests confirmed that the sample contained *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*), *Clostridium bacilli* (*C. bacilli*), *Streptcoccus mutans* (*S. mutans*) and *Staphlococcus aureus* (*Staph. aureus*). AVG showed anti-bacterial property at 100% and 50% concentration ('t' value = 7.504, p-value <0.001). At lower concentration there was no effect against the bacteria. At 100% AVG concentration, zone of inhibition measured was 6.9mm in *A. actinomycetemcomitans*, 6.3mm in *C. bacilli*, 6.8mm in *S. mutans* and 6.6mm in *Staph. aureus*. The standard drugs were also used to compare anti-bacterial property of AVG. Result showed that higher concentration (100%, 50%) of AVG has comparable zone of inhibition with Ofloxacin (5mcg) and Ciprofloxacin (30mcg).

Conclusion: AVG at higher concentration showed anti-bacterial property and can be used as a promising adjunct for oral health care.

Keywords: Anti-microbial, Culture, Herbal

INTRODUCTION

Oral diseases continue to be a major oral health problem and various oral microbes are associated with the development of various oral infections [1]. Anti-microbial therapy is the mainstay line of treatment to treat these infections but recently microbial resistance to the antibiotics has been well documented. This lead to the exploration of natural herbal remedies, which have fewer side effects, for the treatment of various oral diseases and infections [2].

Aloe Vera belongs to Liliacea family which is a cactus-like plant that grows readily in hot, dry climates. Aloe barbadensis miller and Aloe aborescens are grown commercially. The parenchymatous cells, in the fresh leaves of Aloe Vera, secrete a colorless mucilaginous gel that contains 98-99% water and 1-2% active compounds like Aloesin, Aloin, Aloe-emodin, Aloe-mannan, Flavonoids, Saponin, Sterols, Amino acids and Vitamins. Free Anthroquinones and their derivatives like Barbaloin-IO-aloe emodin-9 anthrone, Isobarbaloin and chromones in Aloe Vera leaves exert a strong purgative effect and are potent anti-microbial agents [3].

AVG has various pharmacological actions like antibacterial, anti-fungal, anti-inflammatory, antioxidant, hypoglycemic and immune boosting properties [4-6].

AVG had also been used in dentistry and showed good results. It had been used for treatment of recurrent oral ulceration, oral lichen planus, oral candidiasis, over extraction socket and as an endodontic medicament. Various dentrifices also contain AVG as its constituents because of its medicinal property [7-10].

The aim of the study was to determine the antibacterial and inhibitory activities of various concentration of AVG against oral pathogenic bacteria.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Rungta College of Dental Sciences and Research, Bhilai, Chattisgarh, India. Subgingival calculus was removed using a hand scaler from 20 patients, and then aspiration of periapical and periodontal abscess was done and contents were transferred to thioglycolate broth [Table/Fig-1]. This sample was incubated in Mutans Sanguis agar, blood agar at 37° C for 48 hours in anaerobic jar [Table/Fig-2]. The colonies formed were identified further by gram staining methods, specific culture test (cooked meat media, PDA agar) and biochemical fermentation tests [Indole Methylred Vogesprausker Citrate (IMViC-) utilization] [Table/Fig-3]. Each isolated colonies of identified bacteria's were cultured separately in Muller-Hilton broth and incubated at 37°C for 24 hours.

Preparation of the Extract: Mature fresh leaves of Aloe Vera were washed with fresh, water, their thick epidermis was removed and the solid mucilaginous gel was collected in a sterile container [Table/Fig-4]. A 10 ml of gel was mixed in 100ml 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 AVG was detected using disc diffusion method and micro-dilution and agar dilution method was done to measure minimum Inhibitory Concentration (MIC).

Disc Diffusion Method: The clinical isolate were grown in Mueller-Hinton broth and incubated at 37°C for 24 hours. A 0.1ml of the culture was then poured into sterile petri plate (n=10) and allowed to solidify. The wells were bored (in each plate) with 8mm borer in seeded agar in which 100%, 50%, 25%, 12.5% of AVG extract was poured. After it normalized to room temperature, plates were incubated at 37°C for 24 hours. The zone of inhibition was measured and recorded. Optical density of the culture was adjusted to 0.1 with sterile Mueller-Hinton broth [Table/Fig-5a,b].

Antibiotics [Ciprofloxacin (30mcg) and Ofloxacin (5mcg)] were also poured in different plates against same clinical isolates and zone of inhibition was measured for comparison with AVG [Table/Fig-6a,b].

Minimum Inhibitory Concentration: It was done using micro-broth dilution method [2]. The highest dilution that yielded no single bacterial colony was taken as the MIC.

Extracts were mixed with 2% DMSO to obtain various concentrations of the stock i.e., 100%, 50%, 25%, 12.5%, 6.25%. Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 0.5ml of solution. The tubes were incubated anaerobically at 37°C for 24 hours. Later test dilution was sub-cultured on Mueller Hinton agar and further incubated for 24 hours to check the bacterial growth.

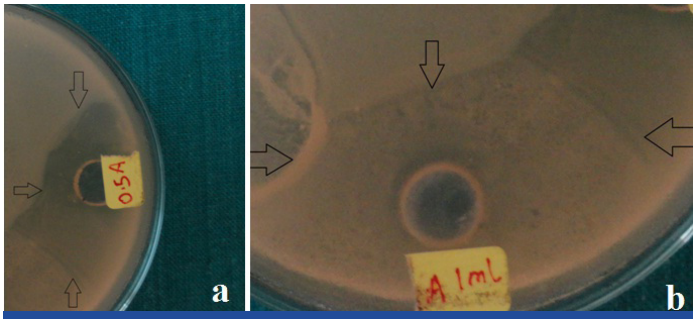
STATISTICAL ANALYSIS

The data were compiled in MS Office Excel. Statistical analysis was done using SPSS version 21 software package (SPSS Statistics for Windows, Version 21.0. Chicago:SPSS Inc.). ANOVA test was performed for intra- and inter-group comparisons. Student 't' test was also performed to compare between different concentration of Aloe Vera 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.

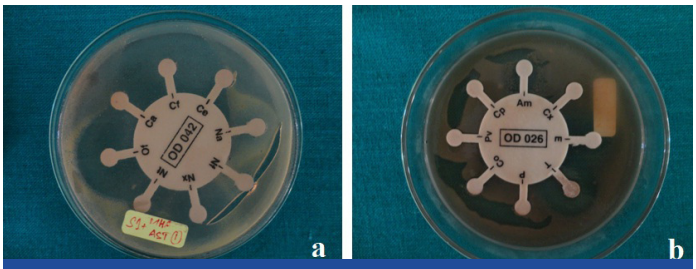
RESULTS

The samples collected were analyzed by staining, specific media culture (cooked meat media, PDA agar) and biochemical tests. The result of which confirmed that it contains strains of *A. actinomycetemcomitans*, *Clostridium bacilli*, *S. mutans* and *Steph. aureus* [Table/Fig-7]. ANOVA test showed that degree of freedom between the groups of oral pathogens was 3 and within the groups of pathogens was 36 and results were statistically significant (p<0.001) [Table/Fig-8]. AVG showed antibacterial property at higher concentrations (100% and 50% concentration, p-value <0.001). At lower concentration (25%, 12.5%) there was no effect against the bacteria [Table/Fig-9]. The 2% DMSO which was used as a dilutent, showed no inhibitory activity on any of these bacteria.

At 100% AVG concentration average zone of inhibition measured was 6.9mm in *A. actinomycetemcomitans*, 6.3mm in *Clostridium bacilli*, 6.8mm in *S. mutans* and 6.6mm in *Staph aureus*. Ciprofloxacin (30mcg) showed 7.4mm zone of inhibition against *A. actinomycetemcomitans*, 7.1 against *Clostridium bacilli*, 6.8mm and 7.3mm against *S. mutans* and *Staph. aureus* respectively. Ofloxacin (5mcg) showed 4.6, 4.8, 5.4, 5.1 zone of inhibition against *A. actinomycetemcomitans*, *Clostridium bacilli*, *S. mutans* and *Staph. aureus* respectively [Table/Fig-10].



[Table/Fig-5a,b]: Zone of Inhibition at 50% and 100% concentration of aloe vera.



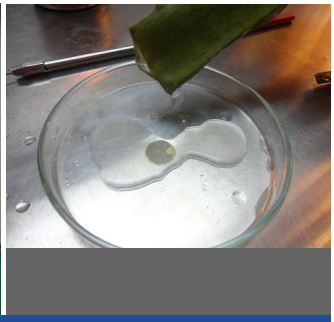
[Table/Fig-6a,b]: Zone of inhibition of antibiotics [ciprofloxacin (30mcg) and ofloxacin (5mcg)].

Bacteria	Gram Reaction	Morpho-logy	Arrange-ment	Other
A. actinomycetemcomitans	(-) Ve	Bacilli	Single	PDA agar culture
Clostridium bacilli	(+) Ve	Bacilli	Single	Cooked meat broth culture
S. mutans	(+) Ve	Cocci	Chain	Horse blood agar
Staph. aureus	(+) Ve	Cocci	Cluster	IMViC fermentation test -Catalase- Positive -MR-VP- Positive -Indole-Negative

[Table/Fig-7]: Isolated oral pathogens identified by gram staining and other test.

Oral Microbes		Degree of Freedom Df	Sig.
A. actinomyetemcomitans	Between Groups	3	< 0.001*
	Within Groups	36	
S. mutans	Between Groups	3	< 0.001*
	Within Groups	36	
C. bacilli	Between Groups	3	< 0.001*
	Within Groups	36	
Staph. aureus	Between Groups	3	< 0.001*
	Within Groups	36	

[Table/Fig-8]: Determining degree of freedom between and within groups of oral pathogens using ANOVA.
* p<0.001; Highly significant for microbes



[Table/Fig-1]: Thioglycolate broth used as transport media. **[Table/Fig-2]:** Anaerobic jar and incubator. **[Table/Fig-3]:** Biochemical (fermentation) tests to identify *Staph aureus*. **[Table/Fig-4]:** Collection of mucilaginous gel of aloe vera.

Concentration of AVG%	N	Mean ± SD	Comparison	't' value	p-value
100%	10	6.88 ± 0.382	100% vs 50%	7.504	< 0.001**
50%	10	5.77 ± 0.241			

[Table/Fig-9]: Anti-bacterial efficacy of aloe vera gel at 100% and 50% concentration.
** p<0.001; Highly significant for microbes

Concentration of AVG%	*A. actinomycetemcomitans* (N=10)	*Clostridium bacilli* (N=10)	*S. mutans* (N=10)	*Staph. aureus* (N=10)
100%	6.9mm	6.3mm	6.8mm	6.6mm
50%	5.8mm	5.4mm	5.6mm	6.1mm
25%	Resistant	Resistant	Resistant	Resistant
12.50%	Resistant	Resistant	Resistant	Resistant
Ciprofloxacin (30mcg)	7.4mm	7.1mm	6.8mm	7.3mm
Ofloxacin (5mcg)	4.6mm	4.8mm	5.4mm	5.1mm
DMSO	0	0	0	0

[Table/Fig-10]: Anti-microbial activity by measuring zone of inhibition.
** p<0.001; Highly significant for microbes

Bacteria	Aloe Vera Gel
A. actinomycetemcomitans	25%
Clostridium bacilli	25%
S. mutans	12.5%
Staph. aureus	12.5%

[Table/Fig-11]: Minimum inhibitory concentrations of aloe vera gel.

MIC of AVG against *A. actinomycetemcomitans*, *Clostridium bacilli*, *S. mutans* and *Staph. aureus* was 25%, 25%, 12.5% and 12.5% respectively [Table/Fig-11].

DISCUSSION

Oral microbial flora is a causative factor for most of the infectious oral diseases. Mostly the periodontal diseases are associated with anaerobic gram negative rods such as *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythus*, *Fusobacterium* species. Antibiotic administration prior to any invasive dental procedure is recommended to prevent from bacteremia and endocarditis by *S. mutans* and other oral bacterial species [2,3].

The main drawback of these anti-microbial agents is that it may have side effect of immediate hypersensitivity reactions, toxicity, tooth staining and some bacteria may show multi-drug resistance [2]. To overcome these drawbacks, various alternative sources are being searched for curing oral diseases. One among these is traditional herbal medicines. Many medicinal plants (fruit of amla, garlic bulbs, ginger, neem, tulsi) and their products are widely used for prevention and treatment of oral infections. These medicinal plants contain natural phytochemicals that have been considered as useful alternatives to synthetic drugs. Among them Aloe Vera is of particular interest and has been used therapeutically for a long time to treat many diseases [2]. Though studies related to its usage in dentistry is limited. So, we tried to check its anti-microbial effectiveness against oral pathogens.

In the present study AVG was found to be effective against both gram positive and gram negative bacteria. Our results were comparable to the study by Bashir A et al., in which AVG was found to be 100% active against all gram negative isolates and 75.3% active against gram positive pathogens [5]. Irshad S et al., found that AVG is effective against *Escherichia coli*, *Bacillus subtilius, Salmonella typhi, Pseudomonas, Klebsiella epidermidis* [11].

By micro-broth dilution method, in the present study it was found that for AVG, MIC was 25% (*A. actinomycetemcomitans*, *C. bacilli*) and 12.5% (*S. mutans*, *Staph. aureus*) and effective zone of inhibition was found at concentration of 100% and 50%. A study reported that concentrations of more than 100mg of Aloe Vera per ml for *S. flexneri* and 25mg of Aloe Vera per ml for *S. pyogenes* were effective in growth inhibition of these organisms [12]. Researchers have reported that the ethanolic extract of Aloe Vera produces a larger zone of growth inhibition (29-30 mm) than the aqueous extract (3-4 mm) against organisms including *Enterococcus bovis* and *Staph. aureus* [13].

Fani M et al., found that *S. mutans* was most sensitive to AVG with a MIC of 12.5%, while *A. actinomycetemcomitans*, *P. gingivalis*, and *B. fragilis* were less sensitive, with a MIC of 25-50% [2]. The results were consistent to our study where *S. mutans* and *Staph. aureus* were most sensitive (12.5%) and *A. actinomycetemcomitans*, *C. bacilli* were less sensitive (25%).

Currently, dentifrices containing AVG are under clinical trials to control the dental plaque and gingivitis. The results of the study suggested that AVG has anti-microbial activity against oral pathogens especially against *S. mutans* which may be attributed due to the presence of active compounds mainly Aloin and Aloe-emodin (anthroquinones) which inhibit protein synthesis by bacterial cells; thus, contributing to its anti-microbial activity. Acemannan exerts indirect anti-bacterial activity mainly by phagocytosis. Aloe Vera, in tooth pastes and dentifrices leads to inhibition of dental caries and reduction of plaque due to its unique anti-bacterial properties [2,14]. This study showed the possibility of the presence of some bioactive components in crude extracts of AVG due to which it has showed strong anti-bacterial effect. Hence, AVG can be used as an alternative anti-bacterial agent to prevent and treat some oral infectious diseases at higher concentration.

LIMITATION

Our study was an in-vitro study, performed over smaller group of patients which could be the limitation of the present research; thus, in future more in-vivo and in-vitro trials should be conducted involving larger samples to test the efficacy of AVG on oral pathogens.

CONCLUSION

This study proved that AVG can be used as an alternative anti-bacterial agent to prevent and treat some oral infectious diseases at higher concentration but in future further clinical trials are required to test the unique antibacterial properties of AVG against various oral pathogens.

ACKNOWLEDGEMENT

We would like to thank and acknowledge Mrs. Priya Jain, MSc. Microbiology, Bilaspur, Chhattisgarh, for her support and guidance.

REFERENCES

[1] Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. *Bull World Health Organ*. 2005;83:661-69.
[2] Fani M, Kohanteb J. Inhibitory activity of aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. *J Oral Sci*. 2012;54:15-21.
[3] Vogler B K, Ernst E. Aloe vera: A systematic review of its clinical effectiveness. *Br J of Gen Pract*. 1994;9:823-28.
[4] Kammoun M, Miladi S, Ali YB, Damak M, Gargouri Y, Bezzine S. In-vitro study of the PLA2 inhibition and antioxidant activities of aloe vera leaf skin extracts. *Lipids Health Dis.* 2011;10:30.
[5] Bashir A, Saeed B, Mujahid TY, Jehan N. Comparative study of antimicrobial activities of aloe vera extracts and antibiotics against isolates from skin infections. *Afr J Biotechnol.* 2011;10:3835-40.
[6] Choonhakarn C. The efficacy of aloevera gel in treatment of oral lichen planus: A randomized control trial. *British J Dermatol.* 2008;158:573-77.
[7] Tanwar R. Aloe vera and its uses in dentistry. *Indian J Dent Adv*. 2011;3(4):656-58.
[8] Saritha V, Anilakumar KR, Khanum F. Antioxidant and antibacterial activity of aloevera gel. *Int J Pharm Biol Arch*. 2010;1:376-84.
[9] Arijani E, Khoswanto C. The use of 90% aloe vera freeze drying as the modulator of collagen density in extraction socket of incicivus Cavia cobaya. *Dent J Maj Ked Gigi*. 2008;41:74-76.

[10] Lardungdee P, Asvanit P, Thunyakitpisal P. Effect of acemannan on the dentinsialophosphoprotein and dentin matrix protein 1 mRNA expressions in primary human pulpal cells. *CU Dent J.* 2008;31:407-14.
[11] Irshad S, Butt M, Yonus H. In-vitro antibacterial activity of aloe barbadensis miller. *Int Res J Pharmaceuticals*. 2011;1:59-64.
[12] Ferro VA. In vitro susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of aloe barbadensis miller. *Antimicrob Agents Chemother.* 2003;47(3):1137–39.
[13] Pandey R, Bradbury F, Cameron P, Shakir E, Rahman SR, Stimson WH. In vitro susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of aloe barbadensis miller. *Appl Biochem Biotechnol*. 2010;160:1356-61.
[14] de Oliveira SM, Torres TC, Pereira SL, Mota OM, Carlos MX. Effect of a dentifrice containing aloe vera on plaque and gingivitis control. A double-blind clinical study in humans. *J Appl Oral Sci*. 2008;16:293-96.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 17, 2016**
Date of Peer Review: **Jul 06, 2016**
Date of Acceptance: **Aug 04, 2016**
Date of Publishing: **Nov 01, 2016**