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Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria

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Abstract: Aloe vera is a medicinal plant with anti-inflammatory, antimicrobial, antidiabetic and immune-boosting properties. In the present study we investigated the inhibitory activities of Aloe vera gel on some cariogenic (*Streptococcus mutans*), periodontopathic (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*) and an opportunistic periodontopathogen (*Bacteroides fragilis*) isolated from patients with dental caries and periodontal diseases. Twenty isolates of each of these bacteria were investigated for their sensitivity to Aloe vera gel using the disk diffusion and microdilution methods. *S. mutans* was the species most sensitive to Aloe vera gel with a MIC of 12.5 µg/ml, while *A. actinomycetemcomitans*, *P. gingivalis*, and *B. fragilis* were less sensitive, with a MIC of 25-50 µg/ml ($P < 0.01$). Based on our present findings it is concluded that Aloe vera gel at optimum concentration could be used as an antiseptic for prevention of dental caries and periodontal diseases. (J Oral Sci 54, 15-21, 2012)

Keywords: *S. mutans*, *A. actinomycetemcomitans*; Aloe vera gel; MIC.

Introduction

Periodontal diseases and dental caries are the two most prevalent oral infections affecting mankind worldwide.

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Endogenous oral bacterial species such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Bacteroides sp.*, *Prevotella sp.*, *Fusobacterium sp.* and their metabolites play major roles in the initiation and progression of these infections (1,2). Effective prevention of these infections can be achieved by mechanical removal of dental plaque by proper tooth brushing and flossing. However, the majority of the population, particularly aged individuals, may not perform mechanical plaque removal sufficiently, and thus antimicrobial mouth rinses such as triclosan and chlorhexidine may be used to limit these two plaque-related oral infections (3). These chemical agents used in the form of either dentifrices or mouth rinses may have undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions (4,5). Although antibiotics are used routinely to prevent systemic infections originating from the oral cavity, they are not recommended for regular prevention of dental plaque formation because of the risk that bacteria will develop resistance to them. During the last decade, extracts or oils of medicinal plants with antimicrobial and anti-inflammatory activity have been used for prevention of various oral infections (6-8). Moreover, *in vitro* inhibitory activity of extracts or oils from various medicinal plants such as garlic (9), *Lippia sidoides* (10), grapefruit seeds (11), and many others (12-15) against cariogenic and periodontopathic bacteria have been documented. Aloe vera is a well known medicinal plant belonging to the *Liliaceae* family. It is a cactus-like plant that grows readily in hot dry climates. The mucilaginous tissue in the center of the Aloe vera leaf (Aloe vera gel) has traditionally been used for treatment of digestive tract disorders, sunburn and wounds. To date, more than

75 active ingredients of the Aloe vera inner gel have been identified. The gel consists of 98-99% water and the remaining 1-2% contains the active compounds, including aloesin, aloin, aloë-emodin, aloëmannan, acemannan, aloëride, naftoquinones, methylchromones, flavonoids, saponin, sterols, amino acids and vitamins. The levels of these compounds in Aloe plants are highly variable according to species and strain, as well as growth conditions. The pharmacological actions of Aloe vera gel as studied *in vitro* and *in vivo* include anti-inflammatory, antibacterial, antioxidant, immune-boosting and hypoglycemic properties (16-20).

In the present study we investigated the inhibitory effects of Aloe vera gel on some periodontopathic, cariogenic and opportunistic pathogenic bacteria isolated from patients with periodontitis and dental caries.

Materials and Methods

Isolation of *Streptococcus mutans* from carious teeth

S. mutans was isolated from carious teeth as described elsewhere (9). Briefly, the extracted teeth were incubated in 10 ml Todd-Hewitt broth (Merck, Germany) at 37°C in the presence of 5% CO₂ for 48 h. A Mitis-Salivarius-Bacitracin-Agar (MSBA) medium was sub-cultured from Todd-Hewitt broth and incubated at 37°C under 5% CO₂ for 72 h. *S. mutans* was then identified by biochemical tests (9). Pure cultures of each clinical isolate of *S. mutans* were prepared on MSBA medium and kept at 4°C until used.

Isolation of periodontopathic bacteria

Patients with either localized aggressive periodontitis or aggressive periodontitis were examined and sampled for isolation of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Bacteroides fragilis*. Subgingival samples were taken from the deepest periodontal pockets (probing depth ≥ 6 mm) by insertion of a sterile paper point (ISO 35, Bocht, Offenburg, Germany). Each sample was inoculated into 5 ml Trypticase soy broth containing hemin and menadione (Becton Dickinson Microbiology Systems) and kept under anaerobic conditions in the presence of 5% CO₂ at 37°C for 48 h. Bacteria from Trypticase soy broth were then sub-cultured on Trypticase-soy-blood-agar (TSBA) plates (composed of 40 g/L Trypticase soy agar/ hemin 5 mg/L, N-acetylmuramic acid 10 mg/L / menadione 0.5 mg/L and sheep blood 50 ml/L) and kept at 37°C under anaerobic conditions in the presence of 5% CO₂ for 72 h. The periodontopathic bacteria were isolated and characterized according to Forbes et al. (21). Pure cultures

of each isolate were prepared on TSBA and kept at 4°C until use.

Preparation of Aloe vera gel

Mature and fresh leaves of Aloe vera approximately 90-100 cm long were washed with fresh water, their thick epidermides were removed, and they were then cut transversely into pieces. The solid mucilaginous, thick straw-colored gel was collected in a sterile container. One hundred grams of the gel was mixed in one liter of 2% dimethyl sulfoxide (DMSO) and kept at 4°C, being used as a stock solution.

Detection of antibacterial activity of Aloe vera inner gel

Two basic methods of antimicrobial susceptibility testing – qualitative and quantitative – are available to diagnostic and research laboratories for detection of bacterial sensitivity or resistance to antimicrobial agents. Disk diffusion, also known as the Kirby-Bauer method, is a qualitative approach that may be prone to some degree of error. However, this technique is used routinely in diagnostic bacteriology laboratories to determine the antibacterial agent sensitivity or resistance of bacteria isolated from patients. Microdilution and agar dilution methodologies are considered quantitative because they can measure the minimum inhibitory concentration (MIC) of an antibacterial agent. The MIC is defined as the lowest concentration of antibiotic or antibacterial agent that inhibits the visible growth of a microorganism. Both quantitative methods are considered the gold standard for susceptibility testing because of their high levels of reproducibility. In this study, we used the disk diffusion and broth microdilution methods to determine the antibacterial activity of Aloe vera gel both qualitatively and quantitatively.

Disk diffusion assay

The antibacterial activities of Aloe vera gel were determined by the standard disk diffusion susceptibility test on solid media (15). MSBA plates were used for *S. mutans* and TSBA plates were used for susceptibility testing of *A. actinomycetemcomitans*, *B. fragilis* and *P. gingivalis*. The cariogenic bacterium *S. mutans* ATCC 25175 and periodontopathic bacteria such as *A. actinomycetemcomitans* ATCC 29523 and *P. gingivalis* ATCC 33277 were used as controls in this study. These strains were maintained anaerobically on TSBA supplemented with 10% defibrinated horse blood and hemin (5 µg/ml; Wako Pure Chemical Industries, Osaka, Japan). A pure bacterial cell suspension of each clinical isolate was

Table 1 Antimicrobial activity of various concentrations of Aloe vera gel on some cariogenic and periodontopathic bacteria by disk diffusion tests

Con. of AVG%	<i>S. mutans</i> n = 20	<i>A. actinomycetemcomitans</i> n = 20	<i>B. fragilis</i> n = 20	<i>P. gingivalis</i> n = 20
100	54	38	40	32
50	30	21	22	17
25	17	12	12	9
12.5	10	< 7	< 7	< 7
6.25	< 7	< 7	< 7	< 7
DMSO 10%	0	0	0	0
Vancomycin	17	0	0	0
Amikacin	0	16	17	16

Numbers are mean diameter of inhibition zones in mm. DMSO = Dimethyl sulfoxide 10% used as negative control and showed no zone of inhibition (0). AVG = Aloe vera gel. Vancomycin (30 µg) and Amikacin (30 µg) were used as reference antimicrobial compounds.

prepared in 5 ml of Todd-Hewitt broth (*S. mutans*) or 5 ml of Trypticase soy broth (*A. actinomycetemcomitans*, *B. fragilis*, *P. gingivalis*) and the suspension turbidity was adjusted to 1.5×10^8 CFU/ml (#0.5 Macfarland). One hundred microliters of this suspension was seeded onto appropriate solid media. A 6-mm-diameter sterile Whatman filter paper No. 5 (round filter Machery-Nagel, D-5160, Doren, Germany) was impregnated with 50 µl of various dilutions of Aloe vera gel and placed on the above culture media, followed by incubation at 37°C under anaerobic conditions for 72 h. The diameter of the zone of growth inhibition around the filter paper was measured in mm and recorded. Sterile filter papers soaked with 50 µl of 10% DMSO, Vancomycin (30 µg), and Amikacin (30 µg) were also used as controls. Any inhibition zone around the filter paper measuring ≤ 7 mm was considered a negative result.

MIC assay

Broth microdilution methods were carried out in 96-well cell culture plates and used to determine the MIC of Aloe vera gel against oral bacterial isolates (22). Todd-Hewitt broth was used for *S. mutans*. Trypticase soy broth containing hemin and menadione was used for *A. actinomycetemcomitans*, *P. gingivalis* and *B. fragilis*. Bacterial cell suspensions of the clinical isolates were prepared in the above liquid media, and their concentrations were adjusted to 10^7 CFU/ml. Two-fold dilutions of Aloe vera gel were prepared in the appropriate broth culture media from the Aloe vera gel stock solution. Aliquots (200 µl) of each dilution of Aloe vera gel were dispensed in 96-well cell culture plates.

One hundred microliters of each bacterial suspension

was added to each well and incubated under anaerobic conditions in a 5% CO₂ atmosphere at 37°C for 48 h. The absorbance was then measured at 595 nm. The highest dilution at which no growth ($D \leq 0.05$) was observed was defined as the MIC.

Statistical analysis

Statistical analysis was performed by the chi-squared and Fisher exact tests using the SPSS software package version 11.5.

Results

The antibacterial activity of Aloe vera gel was initially evaluated by the disk diffusion method using 20 isolates of *S. mutans* as the main causative agent of dental caries and 20 isolates of each of the periodontopathic bacteria, i.e. *A. actinomycetemcomitans* and *P. gingivalis*, and the opportunistic periodontopathogen, *B. fragilis*. Table 1 shows the results of disk diffusion tests on these bacteria. Undiluted Aloe vera gel produced significant growth inhibition zones against all of the oral bacteria tested. The diameter of the growth inhibition zone was directly proportional to the concentration of Aloe vera gel. The zone of inhibition produced by undiluted Aloe vera gel was widest for *S. mutans* (54 mm) and narrowest for *P. gingivalis* (32 mm). At a dilution of 1:8 (12.5%), Aloe vera gel inhibited only *S. mutans*, with an inhibition zone of 10 mm, while all isolates of *A. actinomycetemcomitans*, *P. gingivalis* and *B. fragilis* were resistant to this dilution. None of the above bacteria were sensitive to dilutions of Aloe vera gel of 1:16 or higher. The 10% DMSO used as a diluent showed no inhibitory activity on any of these bacteria. Tables 2 and 3 show the MIC

Table 2 Minimum inhibitory concentrations of Aloe vera gel ($\mu\text{g/ml}$) on some clinically isolated cariogenic and periodontopathic bacteria

	<i>S. mutans</i> <i>n</i> = 20	<i>A. actinomycetemcomitans</i> <i>n</i> = 20	<i>B. fragilis</i> <i>n</i> = 20	<i>P. gingivalis</i> <i>n</i> = 20
AVG	12.5	25	50	25
Vancomycin	30	-	-	-
Amikacin	-	30	30	30

Numbers are mean of MIC of 20 isolates. AVG = Aloe vera gel, - = No growth inhibition

Table 3 Minimum inhibitory concentrations of Aloe vera gel ($\mu\text{g/ml}$) on control isolates of some cariogenic and periodontopathic bacteria

<i>S. mutans</i> ATCC25175	25
<i>A. actinomycetemcomitans</i> ATCC 29523	50
<i>P. gingivalis</i> ATCC 33277	50

Numbers represent mean MIC of 3 experiments.

data for Aloe vera gel against the bacteria used in this study. The mean MIC values for Aloe vera gel measured by the microdilution method against clinical isolates of *S. mutans*, *A. actinomycetemcomitans*, *B. fragilis* and *P. gingivalis* were 12.5, 25, 50, and 25 $\mu\text{g/ml}$, respectively.

Discussion

It is well established that most infectious oral diseases such as dental caries and periodontal disease are linked to the microbial flora of the oral cavity. Dental caries is caused by acidogenic species of bacteria, mainly *S. mutans*, *Lactobacillus*, and *Actinomyces*. These oral bacterial species metabolize sucrose to lactic and other organic acids in dental plaque produced on the surface of the tooth and dissolve calcium phosphate in the enamel, consequently giving rise to dental caries. Periodontal disease is mostly associated with anaerobic Gram-negative rods such as *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythus*, *Bacteroides*, *Prevotella* and *Fusobacterium* species. These periodontopathogens are frequently isolated from periodontal pockets of patients with periodontitis, but up to now it has been unclear which species play the main role in pathogenesis. In this study, *B. fragilis* was used as an example of an opportunistic periodontopathogen, as many reports have documented the isolation of *B. fragilis* from dental plaque or periodontal pockets of patients with periodontitis (23-25).

Prevention of dental caries and periodontal disease can

be achieved by proper and regular tooth-brushing, flossing and rinsing with mouthwashes containing antibacterial agents such as chlorhexidine. Application of antibiotics to eliminate the etiologic agents of these oral infections is not recommended because of the risk of the bacteria developing multi-drug resistance (9,15). However, for prevention of bacteremia and endocarditis by *S. mutans* and other oral bacterial species, antibiotic administration prior to invasive dental procedures is recommended. Chlorhexidine, sodium hypochlorite, cetylpyridinium chloride and amine fluoride are widely used as mouthwashes and irrigating agents that can inhibit the growth of potentially pathogenic oral bacteria. Although these antimicrobial agents are widely used, immediate hypersensitivity reactions, toxicity, tooth staining and other side effects have been reported. Moreover, it has been reported that chlorhexidine and sodium hypochlorite are cytotoxic to human periodontal ligament cells, inhibit protein synthesis, and affect mitochondrial activity, thus having detrimental effects on vital tissues (4,5). Considering the possible development of multidrug-resistant oral bacteria and the side effects of other antibacterial agents there has been a need for different types of agents with better antimicrobial activity and less toxicity. The natural phytochemicals isolated from medicinal plants used in traditional medicine have been considered useful alternatives to synthetic drugs. Many medicinal plants and their products are widely used for prevention and treatment of oral infections (6-15), and among them Aloe vera is of particular interest and has been used therapeutically for a long time (16-20).

Using the agar diffusion method, Pandey and Mishra demonstrated that an ethanolic extract of Aloe vera leaves produced a wider zone of growth inhibition (29-30 mm) than the aqueous extract (3-4 mm) against *Enterococcus bovis* and *Staphylococcus aureus*. An ethanolic leaf extract was also reported to be more effective on Gram-positive than on Gram-negative bacteria, as measured in terms of MIC (20). Ferro et al. (26) also found that *Streptococcus pyogenes* was more sensitive than *Shigella*

flexneri to Aloe vera gel. In the present study, we found that Aloe vera gel exerted strong bactericidal activity against both cariogenic and periodontopathic bacteria, producing growth inhibition zones ranging in width from 32 to 54 mm. The MIC of Aloe vera gel for *S. mutans* was significantly less than that for periodontopathic bacteria, indicating that *S. mutans* was highly sensitive to Aloe vera gel, and higher antibacterial activity was observed against Gram-positive than against Gram-negative bacteria. The lower MIC of Aloe vera gel for Gram-positive bacteria, which has also been reported previously (20,26), might be related to the differences in cell wall structures of the two types of bacteria. Commercially available toothpaste containing Aloe vera extract has been investigated in a clinical trial and compared with fluoridated dentifrices. This toothpaste did not show any significant differences from fluoridated dentifrices for the control and reduction of dental plaque and gingivitis (27). However, the manufacturer has not provided any information on the concentration of Aloe vera extract in their product, and has not mentioned the part of the plant from which the extract was prepared. Although no adverse side effects of Aloe vera have been reported in humans, rare cases of reversible hepatotoxicity (28), contact dermatitis (29), and mild itching have been documented. The data obtained in the present study revealed strong bactericidal activity of Aloe vera gel against some cariogenic and periodontopathic bacteria. This activity is attributed to a number of pharmacologically active compounds including anthraquinones, aloin, aloe-emodin, aloetic acid, anthracene, aloe mannan, aloeride, antranol, chrysophanic acid, resistanol, and saponin (30). Aloin, a bitter-tasting yellow compound, is the C-glycoside derivative of an anthraquinone (31). Aloin and aloe-emodin possess strong antibacterial and antiviral activities as well as laxative, hepatoprotective, and antineoplastic characteristics (32). Aloin and aloe-emodin are the major anthraquinones in aloe plants, and their levels range between 0.1% and 25.5% dry weight in the leaf exudates of 68 Aloe species (33,34). Aloin and aloe-emodin have polyphenolic structures, which can inhibit protein synthesis by bacterial cells, thus explaining their antimicrobial activity. This characteristic may also explain the anti-inflammatory activity of Aloe vera gel (35,36). Saponins, which contain glycoside, are soapy substances that have both cleansing and antiseptic properties (30). It is noteworthy that some compounds like anthraquinones and saponin present in Aloe vera gel have direct antibacterial activities while some other components, such as acemannan, have been considered to exert indirect bactericidal activity through stimulation

of phagocytosis (26,37). Therefore, based on the present data, we believe that the use of Aloe vera gel at optimum concentrations in toothpastes or mouthwashes could be useful for prevention of dental caries and periodontal disease.

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