Assessment of Antifungal Activity of Aloe Vera Toothpaste against Candida Albicans

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Abstract. Herbal products have been the basis for medical therapy and are noted as a form of alternative medicine. Modern medicine also employs the use of many herbal products for therapeutic purposes. Aloe vera is a medicinal herb whose mucilaginous gel is traditionally used to treat different diseases. It consists of the active compounds which include methylchromones, flavonoids, aloesin, aloe-emodin, sterols, amino acids, aloemannan, aloin, acemannan, aloeride, naftoquinones, saponin and vitamins. Aloe vera toothpaste, consequently, has been developed by the Thailand Institute of Scientific and Technological Research (TISTR) to fulfill a strategy of good oral hygiene. The aim of this study was to investigate the antifungal activity of aloe vera toothpaste against Candida albicans in vitro. Toothpaste containing 10% aqueous extract of the aloe vera leaf was prepared by TISTR. The toothpaste solution was prepared by mixing 0.66 g of toothpaste in 1 mL of sterile deionizing water. The suspensions of 10⁷cfu/mL (equivalent to 0.5 McFarland Standard) of Candida albicans ATCC 10231 and 2 clinical isolates were prepared and added to toothpaste solution. After 50 sec, the final solution was transferred to D/E neutralizing broth and cultured at 37 °C for 24-48 h on Sabouraud dextrose agar. Sterile distilled water was used as a control. Colony forming unit (CFU) was counted after incubation. The result showed that aloe vera toothpaste had a significant inhibitory effect against tested candida species compared with a control. In conclusion, result from the present study is scientific information to prove that aloe vera toothpaste could be assigned as natural toothpaste against candida infection. With proper tooth brushing, it is a simple manner, leading to good oral care.

1. Introduction
An overgrowth of Candida in the oral cavity can cause candidosis, which is a common opportunistic infection. The disease is frequently seen in infants and older adults, and also with states of local and systemic immunological suppression such as human immunodeficiency virus (HIV) infection, leukemia, diabetes, systemic chemotherapy, radiation therapy, malnutrition, and the use of systemic corticosteroids and broad-spectrum antimicrobials. Apart from candidosis, Candida species are also indicated to be caries-associated microorganism and are generally used as indicators of caries activity. The high salivary candida carriage has been associated with the severity of caries among children and adults and the most often found are C. albicans [1-4].

Aloe vera is a medicinal herb whose mucilaginous gel is traditionally used to treat different diseases. It consists of the active compounds which include methylchromones, flavonoids, aloesin, aloe-emodin, sterols, amino acids, aloemannan, aloin, acemannan, aloeride, naftoquinones, saponin and vitamins [5].
In dentistry, herbal toothpastes containing aloe vera extract were shown to have significant antimicrobial activity against *Streptococcus* species and *Actinomyces* species as well as an improvement in gingival and plaque index scores of gingivitis patients [6]. In vitro study by Bernardes *et al* [7] demonstrated that aloe vera extract (10% v/v) reduced both growth and germ tube formation by *C. albicans*. In addition, antiplaque and antifungal effectiveness of aloe vera toothpaste was reported among a group of intellectually disabled adolescents [8]. Therefore, the objective of this study was to investigate the antifungal activities of toothpaste containing aloe vera extract against *C. albicans*.

2. Materials and methods

2.1. Toothpaste
Toothpaste containing 10% aqueous extract of the aloe vera leaf was prepared by the Thailand Institute of Scientific and Technological Research (TISTR), Ministry of Science and Technology, Thailand. Toothpaste solution was prepared for the use in further experiment by mixing 0.66 g of toothpaste in 1 mL of sterile deionizing water.

2.2. Microorganisms
Pure cultures of *Candida albicans* ATCC 10231 and 2 strains isolated from patients’ oral lesions were obtained from the culture collection of Oral Microbiology Department, Faculty of Dentistry, Mahidol University. They were maintained in freeze drying condition (lyophilization). Afterwards, they were subcultured on Sabouraud dextrose agar (Difco Laboratories, Detroit, MI) and incubated at 37°C for 24 h. The colonies were inoculated in BHI broth and suspension of 10^7 cfu/mL (equivalent to 0.5 McFarland Standard) of each strain was prepared for antifungal determination.

2.3 Antimicrobial assay
One mL of the prepared candida suspension was added to the toothpaste solution to make a final concentration of 22%, which was equal to the initial concentration of toothpaste in normal tooth brushing [9]. After 50 sec, 10 µl of the solution was transferred to 190 µl of D/E neutralizing broth (Difco Laboratories, Detroit, MI), further 10-fold diluted and cultured at 37 °C for 24-48 h on Sabouraud dextrose agar for the quantitation of *C. albicans*. Sterile distilled water was used as a control. Colony forming unit (CFU) grown on agar plate was counted after incubation.

2.4 Statistical analysis
A statistical analysis was made by Mann-Whitney tests to compare the effect of toothpaste with a control. A p-value of <0.05 was noticed significant. For illustration, the results are presented as means and standard deviations of Log CFU/mL.

All tests were carried out in triplicate on three separate occasions.

3. Results
The results of this investigation showed that aloe vera toothpaste had a significant suppressive effect against all tested candida species compared to control (Table 1).
Table 1. The number of C. albicans after exposure to toothpaste solution

| Toothpaste | C. albicans                |
|------------|----------------------------|
|            | ATCC 10231 | clinical isolate I | clinical isolate II |
| Control    | 5.03±0.15  | 5.18±0.12          | 5.75±0.06          |
| Aloe vera  | 1.63±0.06  | 2.24±1.01          | 4.01±0.15          |

* data expressed as mean±SD of Log colony forming unit (CFU)/mL

4. Discussion
Several chemical agents are commercially available for the treatment of infectious diseases; however, they can shift the balance of oral microbial ecology, cause resistant strains and have undesirable adverse effects including hypersensitivity, immunosuppressive and allergic reactions. As a result, the search for new substances with antimicrobial properties to be used against these microorganisms is necessary. Plant products or natural products have been demonstrated to have an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of microbial growth, and modulation of genetic pathways. Various natural components or phytochemicals isolated from plants are considered as good alternatives. In addition, the World Health Organization (WHO) has estimated that about 80% of the population still depends upon herbal medicines for the treatment of several diseases as a result of simple availability, economic reasons, and less side effects [10].

In dentistry, the aloe vera gel has been tried for healing of aphthous ulcers, alveolar osteitis, and lichen planus lesions. This gel has also been found to have strong bactericidal activity against some cariogenic (*Streptococcus mutans* and *Streptococcus mitis*) and periodontopathic (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Peptostreptococcus anaerobius* and *Bacteroides fragilis*) bacteria [11].

In this study, we developed toothpaste containing aqueous extract of aloe vera leaf and tested against 3 strains of *C. albicans*. All tested strains were incubated with 0.33 g/mL (final concentration) of toothpaste which is equivalent to 22% of the initial toothpaste concentration found in the oral cavity during normal tooth brushing. The result shows the significant suppressive effect of aloe vera toothpaste against all tested strains. The most effective result was demonstrated on the reference strain of *C. albicans* (ATCC 10231). Significant variation was reported on candida activity (e.g. biofilm mass, growth activity) between clinical and laboratory reference strains. The clinical strains were shown to have less biofilm mass and growth activity which has been suggested as a mechanism of antimicrobial resistance [12]. Similar to a previous study, 0.5 – 10% aqueous extract of aloe vera leaf was demonstrated to produce a severe decrease in *C. albicans* growth and the ability to form a germ tube [13]. However, the susceptibility of candida to antimicrobial agents, especially those extracted from plants, may vary due to the geographical and climatic conditions which will affect the phytochemical composition of the plants and its antimicrobial activity.

The components of anthraquinones in aloe vera are thought to be responsible for its antimicrobial activity. There are complex and more than 20 kinds of substances, such as aloin, aloesin and aloe-emodin. The antimicrobial mechanisms of anthraquinonein might be 1) the change in cell wall synthesis and cell membrane permeability which leads to cell damage (by interfering absorption of nutrients from the outside and the leakage of nucleic acids causes the cell deformation), 2) inhibiting the respiratory metabolism (by inhibiting the Krebs cycle of oxidation of sugar and the synthesis of
ATP and NADH in the cell), 3) inhibition of nucleic acid synthesis (by its ability to bind with phosphate group of DNA which affect the replication, transcription and lead to cell death) [14-16].

5. Summary
Toothpaste containing aloe vera extract was demonstrated to have significant inhibitory effect against C. albicans. Data from this study could be scientific evidence to demonstrate that aloe vera toothpaste could be employed as natural antifungal toothpaste. With proper tooth brushing, it is a simple manner, leading to good oral care.

6. References
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