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Development of Butterfly Pea-Aloe Vera Mouthwash with Antimicrobial Activity Against *Streptococcus mutans*

Charuporn Boonkasemsin [1] *Jidapha Arpornrat* [1] *Thidarat Angwarawong* [2] *Poramaporn Klanrit* [3] *Onauma Angwaravong* [4,*]

Research Article

Abstract

Objective: Dental caries is the most common oral health issue in the Thai population, primarily caused by *Streptococcus mutans* (*S. mutans*). Although chlorhexidine (CHX) mouthwash is the gold-standard antibacterial agent for reducing dental plaque and pathogenic microorganisms such as *S. mutans*, it is associated with side effects such as tooth discoloration, altered taste, dry mouth, and oral burning sensations. Consequently, plant-based extracts are increasingly being explored as safer alternatives. Previous studies have demonstrated that both Butterfly pea and Aloe vera possess antibacterial activity against *S. mutans*; however, no study has evaluated the efficacy of a Butterfly pea–Aloe vera solution. Therefore, this study aims to assess the effectiveness of Butterfly pea-Aloe vera solutions against *S. mutans*.

Materials and Methods: To evaluate the antimicrobial effect of Butterfly pea–Aloe vera solutions against *S. mutans*, thirteen groups were tested using a modified broth dilution assay and the drop plate technique. These groups included three Butterfly pea solutions at 400 (BP400), 200 (BP200), and 100 mg/mL (BP100); three Aloe vera solutions at 100 (AV100), 50 (AV50), and 25 mg/mL (AV25); four formulations of Butterfly pea–Aloe vera solutions (BP200_AV50, BP200_AV25, BP100_AV50, BP100_AV25); a bacterial suspension (experimental control); CHX (positive control); and deionized water (DI, negative control). Colony-forming units (CFU/mL) were analyzed using the Kruskal–Wallis and Mann–Whitney U tests (α=0.05).

Results: All Butterfly pea solutions (BP400, BP200, BP100) and combined Butterfly pea–Aloe vera solutions (BP200_AV50, BP200_AV25, BP100_AV50, BP100_AV25) significantly inhibited *S. mutans* compared to the DI group, with no significant difference between the Butterfly pea group and the Butterfly pea–Aloe vera group. All Aloe vera solutions (AV100, AV50, AV25) showed no significant inhibition and performed similarly to the DI group. The CHX group exhibited the highest antibacterial activity.

Conclusion: Butterfly pea solution at 100 mg/mL and the Butterfly pea–Aloe vera solution at BP100_AV25 show promise as natural alternatives to chemical-based mouthwashes for daily caries prevention. Further development will focus on BP100_AV25 as a ready-to-use, chemical-free herbal mouthwash, aiming to harness additional biological properties of the two plants, such as anti-inflammatory, wound-healing, and remineralization effects.

Keywords: Aloe vera/ Antimicrobial activity/ Butterfly pea/ Mouthwash/ *Streptococcus mutans*

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[1] *High School Student, Khon Kaen University Demonstration School, Khon Kaen University.*
[2] *Department of Prosthodontics, Faculty of Dentistry, Khon Kaen University.*
[3] *Division of Oral Diagnosis, Department of Oral Biomedical Sciences, Faculty of Dentistry, Khon Kaen University.*
[4] *Division of Pediatric Dentistry, Department of Preventive Dentistry, Faculty of Dentistry, Khon Kaen University.*
[*] *Corresponding Author*

Introduction

Dental caries is the most prevalent oral infectious disease[1] and remains a significant public health concern in Thailand, with the 2024 National Oral Health Survey reporting high prevalence across all age groups.[2] *Streptococcus mutans* (*S. mutans*), a Gram-positive bacterium, is the primary causative agent, particularly within dental plaque. Dental caries development begins with biofilm formation on the salivary pellicle, which facilitates bacterial adhesion by serving as a receptor for oral bacteria.[1,3] *S. mutans* produces the enzymes glucosyltransferase (GTF) and fructosyltransferase (FTF), which convert sucrose into glucans and fructans, promoting bacterial attachment and plaque development.[4,5] Its ability to metabolize carbohydrates and produce lactic acid contributes to the demineralization of dental enamel. Therefore, once *S. mutans* colonies are established on the tooth surface, they lower the oral pH to critical levels, leading to enamel demineralization and the onset of dental caries.[3] Consequently, preventive strategies aim to reduce or eliminate *S. mutans* colonization.

Chlorhexidine (CHX) mouthwash is the gold standard for reducing dental plaque and pathogenic microorganisms such as *S. mutans*.[3] However, due to its broad-spectrum antimicrobial activity, it is not recommended for regular use. Long-term application can lead to side effects, including oral tissue and tooth discoloration, oral mucosa irritation, taste alteration, dry mouth, burning sensations, and cytotoxicity to human cells, potentially causing cell necrosis.[6-9] These concerns have prompted growing interest in plant extracts as alternative antimicrobial agents with lower toxicity and minimal side effects.[3-5,7,8,10,11]

Butterfly pea (*Clitoria ternatea Linn*.), widely found in Thailand and globally, is traditionally used in cooking as a natural food colorant and in Ayurvedic medicine to treat indigestion, constipation, arthritis, skin conditions, and gastrointestinal disorders.[12,13] Its pharmacological properties include antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, anticancer, and antimicrobial activities,[3,13] attributed to its rich phytochemical content—such as alkaloids, saponins, tannins, flavonoids, and their derivatives including anthocyanins (ternatins), flavonol glycosides, kaempferol glycosides, quercetin glycosides, and myricetin glycosides.[3,5,8,12,13] Studies have shown that Butterfly pea extracts inhibit *S. mutans*, though efficacy varies depending on the extraction method and solvent used. Reported values include a minimum inhibitory concentration (MIC) of 100 µg/mL and a minimum bactericidal concentration (MBC) of 400 µg/mL (aqueous extract),[4] anti-biofilm activity at 100 mg/mL (ethanol extract),[3] and inhibition zones >13 mm at 200 mg/mL and >11 mm at 100 mg/mL (aqueous extract),[4] as well as >9 mm at 50–100 mg/mL (ethanol extract).[3]

Aloe vera, a species of the *Liliaceae* family, has long been used to treat gastrointestinal disorders, sunburns, and wounds.[6] It exhibits various pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, wound-healing, and antineoplastic effects.[6,10,14] These effects are attributed to bioactive compounds such as anthraquinones (and other phenolic compounds, including aloin and aloe-emodin), saponins, acemannan, sterols, amino acids, organic acids, vitamins, and minerals.[7,10,14,15] Aloe vera has gained recognition in oral healthcare, with its extracts incorporated into products like toothpaste and mouthwash.[7,11] Extracts from Aloe vera have demonstrated antimicrobial properties against key bacterial species implicated in dental caries and periodontal infections, including *S. mutans*, *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Bacteroides fragilis* (*B. fragilis*), and *Porphyromonas gingivalis* (*P. gingivalis*).[6,14] Reported MIC values against *S. mutans* include 125 mg/mL (aqueous extraction),[16] 12.5 µg/mL[6] and 12.5%

(dimethyl sulfoxide [DMSO] extract),[15] while MBC values exceeded 750 mg/mL for aqueous extract.[16] Inhibition zones ranged from 6.8 mm at 100% (DMSO extract)[15] to 17 mm at 25 mg/mL (DMSO extract).[6] Variability in outcomes likely reflects differences in extraction methods, solvents, and plant sources.

As mentioned above, studies have shown that Butterfly pea and Aloe vera effectively inhibit *S. mutans*. However, no study has evaluated a Butterfly pea-Aloe vera solution. The objective of this study is to assess the effectiveness of a Butterfly Pea-Aloe Vera solution in inhibiting *S. mutans*. The null hypothesis posits that the Butterfly Pea-Aloe Vera solution will show no significant difference compared to deionized (DI) water and CHX. The expected outcome is to establish it as an alternative to chemical-based antiseptic mouthwashes and as a preliminary guideline for developing fresh, ready-to-use, chemical-free herbal mouthwash products.

Materials and Methods

Preparation of *S. mutans* and growth condition

The *S. mutans* ATCC 25175 DMST 1877 (Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand) stock culture was thawed and cultured on Brain Heart Infusion (BHI) agar (Himedia, Dindori, Nashik, India) using the streak plate technique. The culture was incubated at 37°C in a cell incubator (Shel Lab Incubator, Sheldon Manufacturing, USA) for 48 hours. To ensure purity, colonies from BHI agar were subcultured onto fresh BHI agar and incubated for 24 hours. Subsequently, five colonies of *S. mutans* were inoculated into BHI broth (Himedia) and incubated for 24 hours.

After 24 hours of incubation, the *S. mutans* inoculum suspensions were adjusted to a turbidity of 1×10^8 colony-forming units (CFU/mL) by adjusting the optical density (O.D.) to 0.1 at 600 nm[17] using a spectrophotometer (Genesys 20™; Thermo Fisher Scientific, USA), confirming the desired concentration for further experiments.

Preparation of Butterfly pea solutions and Aloe vera solutions

Butterfly pea and Aloe vera powders (Specialty Natural Product, Chonburi, Thailand) were obtained via aqueous extraction. Solutions were prepared by dissolving the powders in room-temperature DI water using two-fold serial dilutions to final concentrations of 800, 400, 200, 100, and 50 mg/mL for Butterfly pea and 800, 400, 200, 100, 50, 25, 12.5, and 6.25 mg/mL for Aloe vera. Fresh solutions were prepared prior to each experiment. The solutions were not filtered before testing. The sterile tubes used to prepare the Butterfly pea solutions were wrapped in foil to protect them from light.

Optimization of Butterfly pea and Aloe vera concentrations for *S. mutans* inhibition via disc diffusion assay

To evaluate the antimicrobial activity of Butterfly pea and Aloe vera solutions against *S. mutans*, a disc diffusion assay was performed to measure the zone of inhibition. The prepared *S. mutans* suspension was cultured on BHI agar plates using the spread plate technique and a sterilized cotton swab. Subsequently, sterile 6.0 mm filter discs (6-mm-diameter sterile disc, Himedia, PA, USA) were placed on the agar. Twenty microliters of each solution were applied to the discs, with 0.12% CHX (C-20 chlorhexidine antiseptic mouthwash, Chonburi, Thailand) used as a positive control and sterile DI water as the negative control. After 24 hours of incubation, inhibition zones were measured using a digital caliper. Results are presented as the average ± standard deviation of three independent trials.

Antimicrobial activity of Butterfly Pea–Aloe Vera solutions against *S. mutans* via modified broth dilution

The disc diffusion assay identified effective concentrations against *S. mutans* at 400, 200, and 100 mg/mL for Butterfly pea and 100, 50, and 25 mg/mL for Aloe vera. Combined Butterfly Pea–Aloe Vera solutions were prepared by mixing selected concentrations (1:1 ratio) for testing via the modified broth dilution method. Due to excessive viscosity, 400 mg/mL Butterfly pea and 100 mg/mL Aloe vera were excluded from combinations. Thirteen test groups were included, with CHX as the positive control, DI water as the negative control, and *S. mutans* alone as the experimental control (Table 1 and Figure 1A).

In the modified broth dilution method, 500 µL of *S. mutans* suspension (1×10^8 CFU/mL) was incubated with 500 µL of the test solution (Figure 1B). After 24 hours, bacterial growth was assessed using the drop plate technique. Serial ten-fold dilutions (10^0 to 10^{-5}) were prepared in sterile phosphate-buffered saline (PBS), and three 10 µL drops from each dilution were plated on sectioned BHI agar (Figure 1C). Plates were incubated for 24 hours, and colonies were counted from dilutions yielding 3–30 colonies per drop. The total CFU count was averaged from three drops of each countable dilution and calculated as CFU/mL (Equation 1). The experiment was performed in five replicates.

$$\text{Microbial concentration (CFU/mL)} = \frac{\text{Number of colonies count}}{\text{Dilution factor x Volume plate (mL)}} \quad \text{....Equation 1}$$

Statistical analysis

Data normality was tested using the Shapiro-Wilk test. All microbial concentration data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. Statistical analysis was performed using IBM SPSS Statistics 20 (IBM, NY, USA). All statistical tests were conducted at the 0.05 significance level.

Table 1 Experimental group for modified broth dilution

Group	Solution	Abbreviation
1	Bacterial suspension (Experimental control)	BAC
2	Chlorhexidine (Positive control)	CHX
3	Deionized water (Negative control)	DI
4	Butterfly pea solution at 400 mg/mL	BP 400
5	Butterfly pea solution at 200 mg/mL	BP 200
6	Butterfly pea solution at 100 mg/mL	BP100
7	Aloe vera solution at 100 mg/mL	AV100
8	Aloe vera solution at 50 mg/mL	AV50
9	Aloe vera solution at 25 mg/mL	AV25
10	Combine Butterfly pea solution at 200 mg/mL with Aloe vera solution at 50 mg/mL	BP 200_AV 50
11	Combine Butterfly pea solution at 200 mg/mL with Aloe vera solution at 25 mg/mL	BP 200_AV 25
12	Combine Butterfly pea solution at 100 mg/mL with Aloe vera solution at 50 mg/mL	BP 100_AV 50
13	Combine Butterfly pea solution at 100 mg/mL with Aloe vera solution at 50 mg/mL	BP 200_AV 25

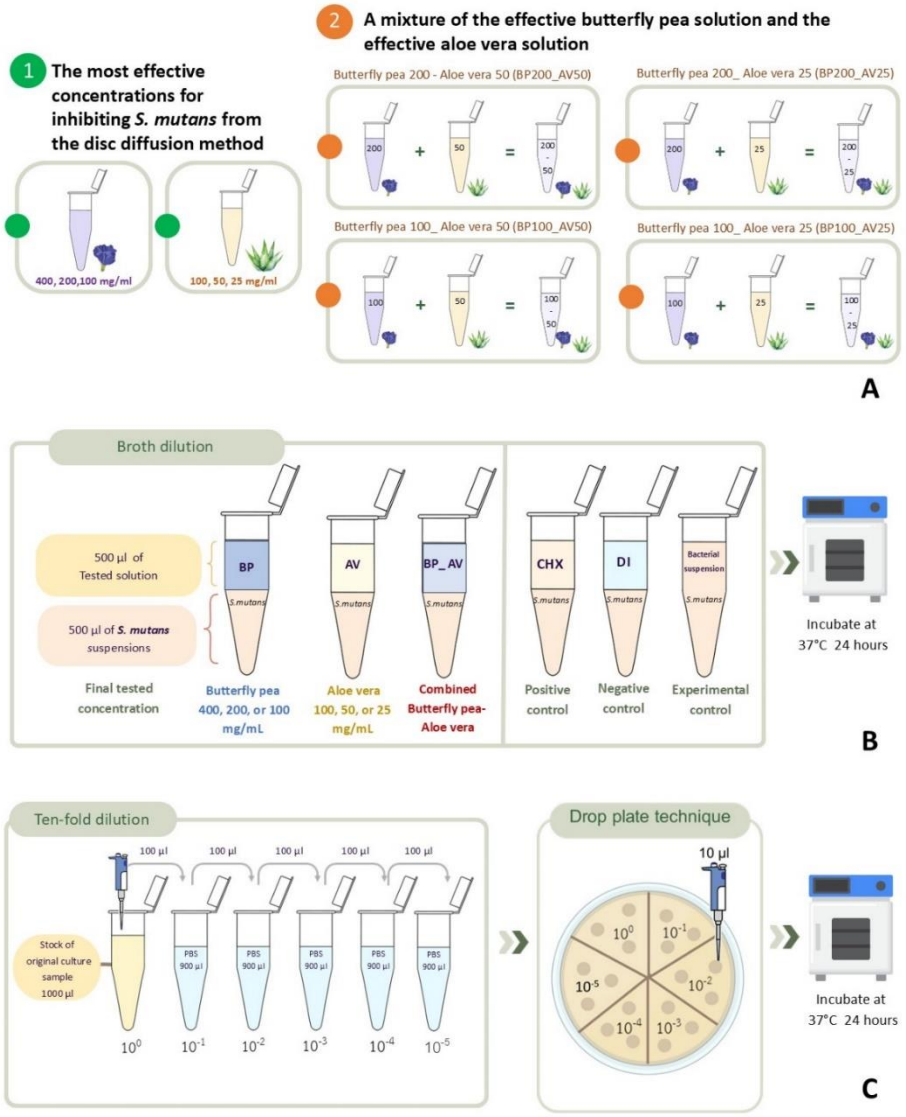

Figure 1 Determination of the efficacy of Butterfly pea-Aloe vera solutions using the broth dilution method. A) Preparation of Butterfly pea solutions, Aloe vera solutions, and Butterfly pea-Aloe vera solutions. B) Modified Broth dilution and incubation for 24 hours. C) Ten-fold dilution and drop plate technique.

Results

The results of the first part, which aimed to determine the optimal concentrations of Butterfly pea and Aloe vera solutions for inhibiting *S. mutans* using the disc diffusion assay, are shown in Tables 2 and 3. The CHX positive control group produced the largest inhibition zone, while the DI water negative control group exhibited no detectable inhibition zone. Butterfly pea solutions at 800, 400, 200, and 100 mg/mL demonstrated similar inhibition zones, in contrast to the 50 mg/mL solution, which showed no detectable inhibition. For Aloe vera solution testing, concentrations of 6.25 and 12.5 mg/mL showed no inhibition, while concentrations ranging from 50 to 800 mg/mL exhibited similar inhibition zones. Therefore, the three lowest effective concentrations—400, 200, and 100 mg/mL for Butterfly pea solution, and 100, 50, and 25 mg/mL for Aloe vera solution—were selected for further study using the modified broth dilution method.

Table 2 Inhibition zones of Butterfly pea solutions

Solution	Mean±S.D.
Chlorhexidine (CHX, Positive control)	21.66±1.06
Deionized water (DI, Negative control)	ND
Butterfly pea solution at 800 mg/mL (BP800)	6.87±0.46
Butterfly pea solution at 400 mg/mL (BP400)	7.76±0.72
Butterfly pea solution at 200 mg/mL (BP200)	8.08±0.69
Butterfly pea solution at 100 mg/mL (BP100)	7.27±0.57
Butterfly pea solution at 50 mg/mL (BP50)	ND

S.D.; Standard deviation, ND; no detection

Table 3 Inhibition zones of Aloe vera solutions

Solution	Mean±S.D.
Chlorhexidine (CHX, Positive control)	22.41±1.06
Deionized water (DI, Negative control)	ND
Aloe vera solution at 800 mg/mL (AV800)	6.73±0.46
Aloe vera solution at 400 mg/mL (AV400)	6.24±0.53
Aloe vera solution at 200 mg/mL (AV200)	6.87±0.27
Aloe vera solution at 100 mg/mL (AV100)	8.15±0.69
Aloe vera solution at 50 mg/mL (AV50)	7.55±0.64
Aloe vera solution at 25 mg/mL (AV25)	7.35±0.13
Aloe vera solution at 12.5 mg/mL (AV12.5)	ND
Aloe vera solution at 6.25 mg/mL (AV6.25)	ND

S.D.; Standard deviation, ND; no detection

The modified broth dilution method was employed to assess the effectiveness of the Butterfly pea-Aloe vera solution in inhibiting *S. mutans* growth. The results demonstrated that the colonies of *S. mutans* on the agar plates varied in number across the different experimental groups, as illustrated in Figure 2. After analyzing the data, the results (as shown in Table 4 and Figure 3) indicated that the DI water group and all Aloe vera solution groups exhibited significantly lower bacterial concentrations compared to the bacteria-only group, with no significant differences observed among these groups. In contrast, all Butterfly pea solution groups and all four Butterfly pea-Aloe vera solution groups demonstrated significantly lower bacterial concentrations than the DI water and Aloe vera groups. Finally, the CHX group was the most effective in inhibiting *S. mutans*, showing a significant difference from the other groups.

Table 4 Microbial concentration (10^6 CFU/mL)

Group	Mean±S.D.	Median	Minimum	Maximum
BAC	76.75±59.89	58.67[a]	20.67	151.40
CHX	0.00±0.00	0.00[b]	0.00	0.00
DI	35.37±28.83	19.00[a]	11.00	68.33
BP 400	4.41±3.87	2.33[c]	1.00	9.67
BP 200	4.48±2.54	3.40[c]	1.67	7.33
BP 100	2.22±2.01	2.00[c]	0.33	4.33
AV 100	38.63±26.38	38.67[a]	4.00	69.00
AV 50	30.37±20.23	30.20[a]	11.33	60.67
AV 25	29.00±27.14	13.67[a]	13.00	60.33
BP 200_AV 50	5.33±2.80	5.67[c]	1.67	8.33
BP 200 _AV 25	3.89±2.44	2.80[c]	2.00	7.67
BP 100_AV 50	4.88±2.42	4.67[c]	2.40	7.67
BP 100 _AV 25	2.17±1.23	1.67[c]	1.33	4.00

Statistical analysis was performed using the Kruskal-Wallis H and Mann-Whitney U tests. Identical letters indicate no significant difference at the same level ($P > 0.05$).

S.D.; Standard deviation, Bac: Bacterial group, CHX: Chlorhexidine group, DI: Deionized water, BP: Butterfly pea, AV: Aloe vera, BP_AV: Combined Butterfly pea-Aloe vera solution in a 1:1 ratio.

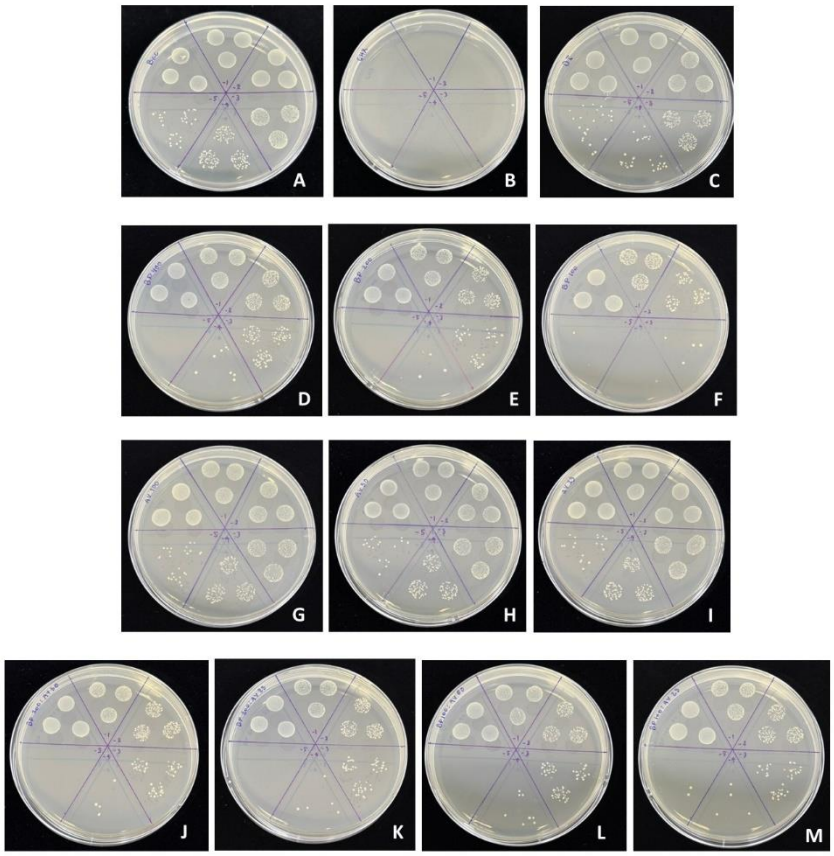

Figure 2 Colonies of *S. mutans* on an agar plate. *S. mutans* colonies on the agar plates from the following groups: Bacterial group (A), Chlorhexidine group (B), Deionized water group (C), Butterfly pea solutions at concentrations of 400 mg/mL (D), 200 mg/mL (E), and 100 mg/mL (F), Aloe vera solutions at concentrations of 100 mg/mL (G), 50 mg/mL (H), and 25 mg/mL (I), as well as Butterfly pea-Aloe vera solutions, namely BP200-AV50 (J), BP200-AV25 (K), BP100-AV50 (L), and BP100-AV25 (M).

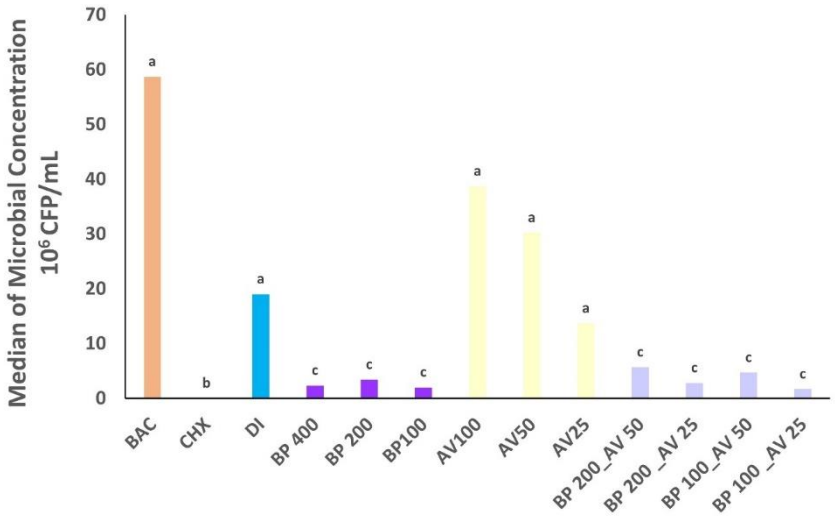

Figure 3 *S. mutans* concentration analysis

Statistical analysis was performed using the Kruskal-Wallis H and Mann-Whitney U tests. Identical letters indicate no significant difference at the same level (P>0.05).

Bac: Bacterial group, CHX: Chlorhexidine group, DI: Deionized water, BP: Butterfly pea, AV: Aloe vera, BP_AV: Combined Butterfly pea-Aloe vera solution in a 1:1 ratio.

Discussion

In this study, all four Butterfly pea–Aloe vera solutions—BP200_AV50, BP200_AV25, BP100_AV50, and BP100_AV25—were found to effectively inhibit *S. mutans* compared to DI water and CHX. Therefore, the null hypothesis was rejected.

Extraction is a crucial step in evaluating medicinal plants, as it separates essential chemical constituents. To preserve bioactive compounds, appropriate extraction methods and solvents must be selected for efficient recovery. Zulkamal et al. found that extraction solvents influence the antimicrobial activity of Butterfly pea, with aqueous extracts exhibiting bactericidal effects against Gram-positive bacteria and bacteriostatic effects against Gram-negative bacteria, while methanolic extracts were bactericidal against both.[18] Methanolic extracts inhibited Gram-positive bacterial growth by 99%, compared to 95% and 79% inhibition by aqueous extracts against Gram-positive and Gram-negative bacteria, respectively.[18] Dental caries are primarily caused by *S. mutans*, a Gram-positive bacterium. Therefore, this study employed aqueous extracts of Butterfly pea and Aloe vera from a certified manufacturer to assess their efficacy against *S. mutans*, as water-based extracts are safe for oral use and can be further developed into a natural herbal mouthwash for future caries prevention.

This study initially employed the disc diffusion (Kirby-Bauer) method to determine the effective concentrations of Butterfly pea and Aloe vera solutions against *S. mutans*, with inhibition zones ≤7 mm considered a negative result.[6] This qualitative method was chosen for its simplicity, low cost, and suitability for routine microbial testing, although it is subject to some degree of error.[6] Despite demonstrating antimicrobial activity, the inhibition zones were small (7.27–8.15 mm) and showed no significant differences among concentrations, likely due to the limited diffusion of the herbal solutions in agar.[7] Given these qualitative limitations, the broth dilution assay was employed for its greater accuracy and quantitative capabilities.[6] The three lowest effective concentrations from the disc diffusion assay—400, 200, and 100 mg/mL for Butterfly pea, and 100, 50, and 25 mg/mL for Aloe vera—were selected for further testing. For combined Butterfly pea-Aloe vera formulations (1:1 ratio), only the 200 and 100 mg/mL Butterfly pea solutions and the 50 and 25 mg/mL Aloe vera solutions were used, as the higher concentrations were excluded due to excessive viscosity, rendering them unsuitable for mouthwash development.

The present findings revealed that Butterfly pea solutions at 400, 200, and 100 mg/mL showed antimicrobial activity against *S. mutans*, consistent with previous studies.[3, 4] This effect may be attributed to bioactive phytochemicals such as flavonoids, saponins, alkaloids, tannins, and anthocyanins (ternatins), which inhibit bacterial growth via multiple mechanisms.[1,3,4,6,8,9,12,19,20] Flavonoids strongly inhibit *S. mutans* by disrupting oral biofilm formation through inactivating GTF enzyme, which is essential for plaque development.[1,9] Biofilm development in *S. mutans* is a sucrose-dependent mechanism, involving GTFs and glucan-binding proteins (GBPs) that synthesize glucans essential for plaque structure. GTFs are key to virulent plaque formation, while Protein antigen c promotes adhesion to the salivary pellicle, contributing to caries progression.[1,3] Flavonoids also disrupt bacterial cell walls and membrane permeability by reducing membrane fluidity, causing fluid imbalance and leakage of proteins, nucleic acids, and ions, ultimately leading to cell death.[19,20] In Gram-positive bacteria, the flavonoid-induced release of Ca^{2+}—crucial for cell wall stability—further compromises structural integrity.[19] These findings align with Zulkamal et al.,

who reported that Butterfly pea exhibited antibacterial activity against *S. mutans* by severely damaging the bacterial surface, causing irregular cell morphology and eventual cell death, as observed via scanning electron microscopy.[4] Saponins exert antibacterial effects by functioning as natural detergents, reducing the surface tension of bacterial cell membranes, which in turn compromises cell wall permeability and impairs bacterial integrity.[6,8] Anthocyanins, the pigments responsible for Butterfly pea's bluish-purple color, also serve as natural dyes,[12] contributing to the aesthetic appeal of potential mouthwash formulations.

Previous studies have shown that Aloe vera contains bioactive components such as saponins and anthraquinones (or other phenolic compounds including aloin and aloe-emodin) that inhibit *S. mutans*.[6,14] However, this study found Aloe vera solutions at 100, 50, and 25 mg/mL ineffective, with results similar to DI water. Although the experimental data indicated that higher concentrations of Aloe vera were associated with increased microbial concentrations, statistical analysis revealed no significant difference ($p > 0.05$). These findings contrast with Majid et al., who reported reduced *S. mutans* levels in plaque after rinsing with a diluted Aloe vera extract.[14] Notably, their extract was ethanol-based and derived from the peel, with ethanol comprising one-fourth of the final solution (1:4 extract:water).[14] Fani and Kohanteb also reported that a 2% DMSO extract of Aloe vera gel exhibited inhibitory effects on *S. mutans*, as measured by disc diffusion and microdilution methods.[6] Variations in outcomes may be due to differences in the source of Aloe vera—such as species, strain, and growth conditions—as well as extraction methods, solvents, and plant parts (gel vs. peel) used, all of which influence the concentration of bioactive compounds.[6, 16]

This study is the first to evaluate the efficacy of combined Butterfly pea–Aloe vera solutions against *S. mutans*. Formulations BP200_AV50, BP200_AV25, BP100_AV50, and BP100_AV25 demonstrated statistically significant antimicrobial activity compared to DI water. However, no significant differences were observed when compared to Butterfly pea solutions at 400, 200, and 100 mg/mL. This result is consistent with the finding that Aloe vera at 50 and 25 mg/mL had no inhibitory effect on *S. mutans*. Although Aloe vera alone was ineffective, the present study proposes further development of the Butterfly pea–Aloe vera solution as a natural herbal mouthwash, which may offer advantages over using Butterfly pea alone. This is supported by previous studies highlighting Aloe vera's wound-healing, anti-inflammatory, and remineralization properties, as well as its benefits in reducing gingival inflammation, treating oral ulcers, relieving pain, and moisturizing wounds.[10, 21-24] All combined Butterfly pea–Aloe vera solutions in this study demonstrated significantly lower antimicrobial efficacy against *S. mutans* compared to CHX. Although CHX is effective in reducing *S. mutans*, its broad-spectrum antimicrobial activity makes it unsuitable for regular use. Moreover, long-term application can result in various side effects, including tooth and oral tissue discoloration, mucosal irritation, taste alteration, dry mouth, burning sensations, and cytotoxicity to human cells, potentially leading to cell necrosis.[6-9] Therefore, the Butterfly pea–Aloe vera solution may serve as a suitable prophylactic option for individuals at high risk of dental caries, plaque-induced gingivitis, and orthodontic patients prone to white spot lesions around brackets[25] and traumatic oral ulcers affecting the gingival and buccal mucosa—common side effects of orthodontic treatment.[23]

The present study showed that the 100 mg/mL Butterfly pea solution and the BP100_AV25 formulation were the lowest concentrations to demonstrate significant efficacy against *S. mutans*, with no significant difference compared to higher concentrations of both Butterfly pea solutions and combined Butterfly pea–Aloe vera solutions. These effective lower concentrations do not result in excessive viscosity, making them suitable for use as mouthwash, and may serve as natural alternatives for daily use in preventing dental caries, potentially replacing chemical-based mouthwashes containing CHX. However, as previously mentioned, the Butterfly pea–Aloe vera formulation BP100_AV25 is suitable for future development as a ready-to-use, chemical-free herbal mouthwash and could be particularly beneficial for individuals seeking natural oral care options or those sensitive to synthetic ingredients commonly found in conventional mouthwashes. In this study, Butterfly pea and Aloe vera powders were obtained through aqueous extraction with clearly defined expiration dates. These powders can be dissolved in water at specified amounts to achieve the desired concentration and used immediately as a mouthwash. This preparation method allows for a chemical-free formulation by eliminating the need for preservatives and additional solvents such as alcohol. Furthermore, because Butterfly pea extract is naturally pigmented, there is no need to add artificial coloring agents to the formulation.

The limitation of this study is its laboratory-based design, which does not fully replicate the complexities of the human oral cavity. Additionally, the study focused only on *S. mutans*, while other bacteria like *Lactobacillus*, *Staphylococcus aureus*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *B. fragilis*, and *P. gingivalis* also play a role in dental caries and periodontal disease.[3, 6, 7, 26] Further research is needed to assess the antimicrobial activity of the Butterfly pea-Aloe vera solutions against these bacteria. Additionally, analyzing the bioactive components and isolating the specific compounds responsible for the antimicrobial effects of Butterfly pea and Aloe vera, as well as understanding their mechanisms, is essential. Although minimal side effects have been reported for both herbs,[10,12] toxicity assessments are needed to confirm their safety in mouthwash formulations. Finally, exploring other biological properties such as anti-inflammatory, wound-healing, and remineralization effects will help support the development of a comprehensive herbal mouthwash.

Conclusion

Based on this *in vitro* study, Butterfly pea solutions at 400, 200, and 100 mg/mL, along with all four Butterfly pea–Aloe vera solutions (BP200_AV50, BP200_AV25, BP100_AV50, and BP100_AV25), exhibited significant inhibitory effects against *S. mutans* compared to DI water. In contrast, Aloe vera solutions at 100, 50, and 25 mg/mL showed no significant antimicrobial activity. CHX mouthwash demonstrated the highest antibacterial efficacy, significantly outperforming all other groups.

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Corresponding Author

Onauma Angwaravong
Division of Pediatric Dentistry,
Department of Preventive Dentistry,
Faculty of Dentistry, Khon Kaen University,
Amphur Muang, Khon Kaen, 40002.
Tel. : +66 43 204 208
Email : onaang@kku.ac.th

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การพัฒนาน้ำยาบ้วนปากอัญชันว่านหางจระเข้ที่มีฤทธิ์ต้านจุลชีพ *Streptococcus mutans*

จารุพร บุญเกษมสิน [1] จิดาภา อาภรณ์รัตน์ [1] ธิดารัตน์ อังวราวงศ์ [2] ปรมาภรณ์ กลั่นฤทธิ์ [3] อรอุมา อังวราวงศ์ [4,]*

บทความวิจัย

บทคัดย่อ

วัตถุประสงค์: โรคฟันผุเป็นปัญหาสุขภาพช่องปากที่พบได้บ่อยที่สุดในประชากรไทย โดยมีสาเหตุหลักจากแบคทีเรียสเตรปโตค็อกคัส มิวแทนส์ แม้ว่าน้ำยาบ้วนปากคลอร์เฮกซิดีนเป็นสารต้านเชื้อแบคทีเรียมาตรฐานที่สามารถลดคราบจุลินทรีย์และจุลชีพก่อโรคในช่องปาก เช่น แบคทีเรียสเตรปโตค็อกคัส มิวแทนส์ ได้ แต่การใช้อาจก่อให้เกิดผลข้างเคียง เช่น ฟันเปลี่ยนสี การรับรสเปลี่ยนแปลง ปากแห้ง และรู้สึกแสบร้อนในช่องปาก ด้วยเหตุนี้การใช้สารสกัดจากพืชจึงได้รับความสนใจมากขึ้น ในฐานะทางเลือกที่ปลอดภัยกว่า งานวิจัยก่อนหน้านี้พบว่า อัญชันและว่านหางจระเข้มีฤทธิ์ต้านเชื้อสเตรปโตค็อกคัส มิวแทนส์ อย่างไรก็ตาม ยังไม่มีการศึกษาถึงประสิทธิภาพของสารละลายอัญชันว่านหางจระเข้ ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อประเมินประสิทธิภาพของน้ำยาบ้วนปากดอกอัญชันว่านหางจระเข้ต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์

วัสดุอุปกรณ์และวิธีการ: ประเมินฤทธิ์ต้านจุลชีพของสารละลายอัญชันและว่านหางจระเข้ต่อเชื้อสเตรปโตค็อกคัส มิวแทนส์ด้วยวิธี modified broth dilution assay และเทคนิค drop plate โดยทำการทดสอบทั้งหมด 13 กลุ่ม คือ สารละลายอัญชัน 3 ความเข้มข้น ได้แก่ 400 (BP400), 200 (BP200) และ 100 (BP100) มก./มล. สารละลายว่านหางจระเข้ 3 ความเข้มข้น ได้แก่ 100 (AV100), 50 (AV50) และ 25 (AV25) มก./มล. สารสะลายอัญชันว่านหางจระเข้ 4 สูตร (BP200_AV50, BP200_AV25, BP100_AV50, BP100_AV25) สารแขวนลอยของเชื้อแบคทีเรีย (ตัวควบคุมเชิงทดลอง) คลอร์เฮกซิดีน (ตัวควบคุมเชิงบวก) และน้ำปราศจากไอออน (ตัวควบคุมเชิงลบ) จากนั้นเอาจำนวนโคโลนีของแบคทีเรียต่อมิลลิลิตร มาวิเคราะห์ด้วยสถิติการทดสอบครัสคัล-วอลลิส และแมน-วิทนีย์ ยู โดยกำหนดระดับนัยสำคัญทางสถิติที่ α=0.05

ผล: สารละลายอัญชันทั้งหมด (BP400, BP200, BP100) และสารละลายอัญชันว่านหางจระเข้ทั้งหมด (BP200_AV50, BP200_AV25, BP100_AV50, BP100_AV25) สามารถยับยั้งเชื้อสเตรปโตค็อกคัส มิวแทนส์ได้อย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มน้ำปราศจากไอออน และไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่มสารละลายอัญชันกับกลุ่มสารละลายอัญชันว่านหางจระเข้ นอกจากนี้ยังพบว่าสารละลายว่านหางจระเข้ (AV100, AV50, AV25) ไม่แสดงฤทธิ์ในการยับยั้งอย่างมีนัยสำคัญและมีผลใกล้เคียงกับกลุ่มน้ำปราศจากไอออน ขณะที่กลุ่มคลอร์เฮกซิดีนแสดงฤทธิ์ต้านแบคทีเรียสูงที่สุด

บทสรุป: สารละลายอัญชันที่ความเข้มข้น 100 มก./มล. และสารละลายอัญชันว่านหางจระเข้สูตร BP100_AV25 แสดงศักยภาพในการเป็นทางเลือกจากธรรมชาติแทนน้ำยาบ้วนปากที่มีสารเคมีในการใช้งานประจำวันเพื่อป้องกันฟันผุ การพัฒนาต่อไปจะมุ่งเน้นการสร้าง BP100_AV25 ให้เป็นน้ำยาบ้วนปากสมุนไพรพร้อมใช้ ที่ปราศจากสารเคมี โดยหวังผลจากคุณสมบัติทางชีวภาพอื่นเพิ่มเติมจากพืชทั้งสองชนิด เช่น การต้านการอักเสบ การสมานแผล และการคืนแร่ธาตุ

คำไขรหัส: น้ำยาบ้วนปาก/ ฤทธิ์ต้านจุลชีพ/ ว่านหางจระเข้/ สเตรปโตค็อกคัส มิวแทนส์/ อัญชัน

ผู้ประพันธ์บรรณกิจ

อรอุมา อังวราวงศ์
แขนงวิชาทันตกรรมสำหรับเด็ก สาขาวิชาทันตกรรมป้องกัน
คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
อำเภอเมือง จังหวัดขอนแก่น
โทรศัพท์ : 043 204 208
จดหมายอิเล็กทรอนิกส์ : onaang@kku.ac.th

[1] นักเรียนมัธยมปลาย โรงเรียนสาธิตมหาวิทยาลัยขอนแก่น มหาวิทยาลัยขอนแก่น
[2] สาขาวิชาทันตกรรมประดิษฐ์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
[3] แขนงวิชาวินิจฉัยโรคช่องปาก สาขาชีวเวชศาสตร์ช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
[4] แขนงวิชาทันตกรรมสำหรับเด็ก สาขาวิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
[*] ผู้ประพันธ์บรรณกิจ