

Development of an oral spray formulated with stingless bee propolis and mangosteen pericarp extracts

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ABSTRACT

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Received: 24 April 2025
Revised: 25 August 2025
Accepted: 21 September 2025
Published: 18 May 2026

Citation:
Charernsriwilaiwat, N.,
Wijitpiyakul, K., Woradechakul,
K., Thaitrong, R., & Subongkot,
T. (2026). Development of an
oral spray formulated with
stingless bee propolis and
mangosteen pericarp extracts.
*Science, Engineering and Health
Studies*, 20, 26050004.

This study developed an alcohol-free, sugar-free oral spray containing stingless bee propolis (SBP) and mangosteen pericarp (MP) extracts with desirable physicochemical properties and stability. The formulations were evaluated for pH, viscosity, and SBP and MP extract content using high-performance liquid chromatography. Antioxidant and antimicrobial activities were assessed using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay and antibacterial testing against *Streptococcus pyogenes*. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was employed to assess the cytotoxicity of the spray to human gingival fibroblasts. Microbial contamination and stability were also examined. The formulations, which contained up to 25 mg of each extract, had a pH range of 6.02–6.54 and viscosities of 0.46–1.19 mPa·s, demonstrating Newtonian flow. The SBP and MP extract contents of the formulation ranged from 99.94% to 102.27%. The antioxidant inhibition rates were 62.04%–84.62%, while the antimicrobial activity was observed at a 50% concentration. Cytotoxicity tests confirmed over 50% cell viability in oral mucosal cells after 15 seconds of exposure. Microbial contamination met safety standards, and the formulations remained stable for 6 months under both normal and accelerated conditions, with the α -mangostin content ranging between 96.12% and 98.76%. This study successfully developed a novel stable oral spray incorporating MP and SBP extracts with favorable organoleptic properties, antioxidant potential, and antimicrobial efficacy, indicating its potential for oral health applications.

Keywords: oral spray formulation; stingless bee propolis extract; mangosteen pericarp extract; antioxidant activity; antimicrobial properties

1. INTRODUCTION

Inflammation of the throat and tonsils is a common health concern, often leading to symptoms such as sore throat, throat discomfort, and irritation. This condition typically arises from viral or bacterial infections, or noninfectious factors. Viral infections generally present with clear nasal discharge and mild tonsillar redness without pus. In

contrast, bacterial infections, most notably caused by Group A *Streptococcus*, account for 20%–30% of cases in children and approximately 5%–15% in adults, and are associated with swollen tonsils and yellowish-white exudate (Shulman et al., 2012). Noninfectious causes include gastroesophageal reflux disease (GERD), smoking, allergen exposure, and environmental conditions, such as cold, humid air. Treatment approaches vary depending on

the etiology. For bacterial infections, antibiotics, such as penicillin V, remain the first-line treatment because of their efficacy and low resistance profile. For patients allergic to penicillin, macrolides, such as azithromycin, are recommended alternatives. Symptomatic treatment focuses on relieving pain and inflammation and may include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or herbal medicines. Furthermore, the use of lozenges and herbal throat sprays containing natural extracts has become increasingly popular, particularly among patients seeking complementary and alternative therapies (CDC, 2022).

Liquid pharmaceutical preparations, such as mouth and throat sprays, have gained increasing popularity due to their ease of use, portability, rapid onset of action, and suitability for frequent application. These features make them particularly advantageous for managing symptoms such as sore throat, irritation, and inflammation in the oral and pharyngeal regions. Furthermore, these formulations facilitate localized drug delivery, minimizing systemic side effects and increasing patient compliance. In recent years, consumer preferences have shifted significantly toward products that incorporate natural ingredients (Ekor, 2014). Natural extracts, such as propolis, chamomile, ginger, and licorice, have been widely incorporated into mouth and throat sprays because of their well-documented anti-inflammatory, antimicrobial, and soothing properties (Kuropatnicki et al., 2013).

Stingless bees are eusocial insects categorized into five genera: *Melipona*, *Trigona*, *Meliponula*, *Dactylurina*, and *Lestrimelitta*. Found primarily in Africa and Asia, stingless bees are crucial in pollination and produce propolis similar to that of honeybees, particularly *Trigona* spp. (Choudhari et al., 2012; da Silva et al., 2013; Paulino et al., 2008). The chemical composition of stingless bee propolis varies by species and habitat and consists of compounds such as flavones, flavanones, terpenes, and phenolics (Athikomkulchai et al., 2013; Rao et al., 2016). Propolis created by stingless bees from mangosteen orchards contains α -mangostin, a compound with potent wound-healing properties, alongside flavonoids and phenolics, and exhibits anti-tumor, antioxidant, anti-inflammatory, and antimicrobial activities (Vongsak et al., 2015).

Previous studies exploring the development of oral care products incorporating SBP from mangosteen orchards confirm its modest antioxidant and antibacterial effects (Prasertrungpanich et al., 2017). Commercial mouth and throat sprays frequently contain honeybee propolis, as well as other natural ingredients, such as chamomile, fingerroot (*Boesenbergia rotunda*), *Andrographis paniculata*, licorice root, ginger, and essential oils. However, products integrating extracts from both SBP and mangosteen pericarp (MP) are scarce.

This study developed an oral spray formulation containing extracts from SBP and MP, both rich in α -mangostin. The combined extracts are hypothesized to synergistically enhance antioxidant activity and inhibit *Staphylococcus pyogenes*. Furthermore, the study assessed the formulation's physicochemical properties, antioxidant capacity, antibacterial efficacy against *S. pyogenes*, microbial contamination, and product stability.

2. MATERIALS AND METHODS

2.1 Materials

SBP was sourced from a local farmer in Patthawi, Makhm District, Chanthaburi Province, Thailand. Extracts from the mangosteen pericarp (MP) were collected from a fruit orchard in the same province. Chemical reagents, such as acetonitrile and *ortho*-phosphoric acid (high-performance liquid chromatography [HPLC] grade, purity > 98%), were acquired from RCI Labscan, Bangkok, Thailand. Human gingival fibroblast cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Dimethyl sulfoxide (DMSO) was sourced from BDH Laboratories (United Kingdom). Cell culture supplies, including Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, and penicillin-streptomycin, were purchased from Gibco BRL (Rockville, MD, USA). All other chemicals and solvents used were of analytical grade and commercially available.

2.2 Extraction of SBP and MP

The SBP was cut into small pieces, washed with distilled water, and dried in a hot-air oven at 40°C for 24 h. Dried propolis (4 g) was macerated in 40 mL of ethanol (1:10, w/v) in 50-mL tubes and sonicated at 40°C $\pm$ 5°C for 1 h. The mixtures were centrifuged twice at 5,000 rpm at 20°C for 20 min. The supernatants were collected and evaporated under a fume hood at room temperature overnight. The dried extract was partitioned with hexane (4 $\times$ 100 mL) to remove wax, and the final extract was air-dried under a fume hood for 48 h (Pothitirat et al., 2010).

Fresh MP was cleaned, and the inner flesh was removed. The pericarps were cut into small pieces and dried in a hot-air oven at 50°C for 72 h. The dried pericarps were ground into powder, passed through a 20-mesh sieve, and stored in light-protected, airtight containers. The powder (50 g) was extracted using a Soxhlet apparatus with 500 mL of 95% ethanol at 78.1°C for 3 h. The crude extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 50°C (Vongsak et al., 2015). The percentage yield of the extracts was calculated using Equation (1):

$$\text{Yield (\%)} = \left(\frac{\text{Weight of dried extract (g)}}{\text{Initial weight of raw material (g)}} \right) \times 100 \quad (1)$$

Because both SBP and MP extracts originated from the same mangosteen orchard, they were expected to contain α -mangostin. The α -mangostin content in both extracts was analyzed by high-performance liquid chromatography (HPLC; Agilent Technologies, USA). The analysis was performed using a VertiSep® AQS C18 column (250 mm $\times$ 4.6 mm, 5 μ m) with a C18 guard column. The method was adapted from Pothitirat et al. (2009) with minor modifications. The mobile phase consisted of acetonitrile (solvent A) and 0.1% v/v *ortho*-phosphoric acid (solvent B), with a flow rate of 1 mL/min at ambient temperature. The gradient elution was programmed as follows: 70% A (0–15 min), 70%–75% A (15–18 min), 75%–80% A (18–19 min), held at 80% A (19–25 min), and returned to 70% A (25–26 min). Detection of α -mangostin was performed at a wavelength of 320 nm using a UV-visible detector (Pothitirat et al., 2009).

The antioxidant activity of the SBP and MP extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A 100- μ L aliquot of 200 μ M DPPH in ethanol was added to 100 μ L of the extract solution (dissolved and diluted in ethanol to the desired concentrations). The mixture was incubated at 37°C for 30 min, and the absorbance was measured at 520 nm using a microplate reader (FLUOstar® Omega, BMG Labtech, Germany). The percentage of radical scavenging activity was calculated using Equation (2):

$$\text{Inhibition (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (2)$$

where A_{sample} is the absorbance of the sample, and A_{control} is the absorbance of the DPPH solution without extract. The IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH radicals, was determined by linear regression analysis of the dose-response curve (% inhibition vs. extract concentration in μ g/mL).

The antibacterial activity of SBP and MP extracts against *S. pyogenes* was evaluated using the macrodilution broth method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) following standard protocols (Wiegand et al., 2008). Briefly, *S. pyogenes* colonies cultured for 24 h were suspended in brain heart infusion (BHI) broth and incubated at 37°C in a shaking incubator. Extracts were prepared in DMSO at an initial concentration of 80 μ g/mL. Two-fold serial dilutions were performed in BHI broth in tubes 1–6, while tube 7 served as a bacterial growth control and tube 8 as a media control. The bacterial inoculum was adjusted to

approximately 1×10^6 CFU/mL using McFarland standard No. 0.5 or by spectrophotometric absorbance at 625 nm (optical density [OD] = 0.08–0.13), followed by a 1:10 dilution. Then, 500 μ L of the adjusted inoculum was added to tubes 1–7 to a final bacterial concentration of 5×10^5 CFU/mL. After incubation at 37°C for 16–24 h, the MIC was recorded as the lowest extract concentration inhibiting visible growth. Tubes without turbidity were subcultured onto blood agar plates and incubated at 37°C for 18–24 h to determine the MBC, defined as the lowest concentration showing no colony growth.

2.3 Formulation of oral spray

The formulations of the oral spray containing various concentrations of the SBP and MP extracts are shown in Table 1. We selected the concentration of the combined extracts that inhibited *S. pyogenes* (50 mg/100 mL, Formulation M4) and subsequently reduced the concentration of each extract to 10 and 5 mg/100 mL to test the hypothesis of synergistic activity. To prepare the formulations, 5, 10, or 25 mg of each extract (SBP and MP) were weighed and dissolved in propylene glycol in a beaker at 40°C. Separately, purified water equivalent to $\frac{3}{4}$ of the total volume was heated to 90°C in another beaker, and poloxamer 407 was added with continuous stirring until completely dissolved. After cooling to room temperature, sucralose, propylparaben, methylparaben, menthol, honey, bubble gum flavor, peppermint oil, spearmint oil, and tartrazine were added to the extract solution and mixed well. The extract mixture was then slowly added to the aqueous base under constant stirring. Finally, the solution was adjusted to the desired final volume with purified water.

Table 1. Formulation of the oral spray containing SBP and MP extracts

Formulation	Amount (g)			
	M1	M2	M3	M4
SBP extract	0	0.005	0.01	0.025
MP extract	0	0.005	0.01	0.025
Propylene glycol	10.4	10.4	10.4	10.4
Poloxamer P407	4	4	4	4
Menthol	0.4	0.4	0.4	0.4
Sucralose	0.1	0.1	0.1	0.1
Honey	0.5	0.5	0.5	0.5
Bubble gum flavor	0.04	0.04	0.04	0.04
Peppermint oil	0.04	0.04	0.04	0.04
Spearmint oil	0.02	0.02	0.02	0.02
Methyl paraben	0.1	0.1	0.1	0.1
Propyl paraben	0.02	0.02	0.02	0.02
Tartrazine	0.02	0.02	0.02	0.02
Purified water added	100	100	100	100

2.4 Characterization of the oral spray containing SBP and MP extracts

Various physicochemical parameters of the oral spray formulations were evaluated. The pH was measured using a calibrated pH meter (Mettler Toledo, Thailand), and each measurement was conducted in triplicate. Viscosity was assessed using a rheometer (KNX2100, Kinexus Pro, Malvern, UK) across a shear rate range of 0.1–100 s^{-1} at 37°C, also measured in triplicate. The quantification of the SBP and MP extracts in the formulations was evaluated by

HPLC, using α -mangostin as a marker compound. The content percentage was calculated using Equation (3):

$$\text{Content (\%)} = (C_a / C_t) \times 100 \quad (3)$$

where C_a is the amount of SBP and MP extracts quantified post-formulation, and C_t is the initial quantity incorporated into the formulation.

The in vitro antioxidant activity of the spray formulations was examined using the DPPH assay as described in section 2.2.



2.5 Antibacterial activity

The antimicrobial activity against *S. pyogenes* of the oral spray formulation was tested using the pour plate method. The oral spray was prepared without preservatives and sterilized by filtration through a 0.22- μ m membrane. The *S. pyogenes* strain was prepared to an approximately 1×10^6 CFU/mL concentration by adjusting its turbidity to McFarland standard No. 0.5 or by measuring the absorbance at 625 nm to an OD of 0.08–0.13. A 500- μ L aliquot of the prepared bacterial suspension was mixed with 500 μ L of the oral spray formulation in a microcentrifuge tube, resulting in a 50% product concentration. A negative control with a sterile medium and a positive control with amoxicillin at 10 μ g/mL were also prepared. All samples were incubated at 37°C for 16–24 h. The absorbance at 550 nm (OD550) of each sample was measured, after which 100 μ L of the clear liquid from each tube was plated onto blood agar plates, which were then incubated at 37°C for 18–24 h. The number of colonies that formed was counted and recorded (Cappuccino & Welsh, 2017).

2.6 Cytotoxicity

The cytotoxicity of the oral spray formulation was evaluated in human gingival fibroblasts using a modified MTT assay according to the ISO 10993-5 guidelines. The oral spray was first sterilized using UV irradiation for 1 h. Human gingival fibroblasts were cultured in DMEM supplemented with 10% FBS and seeded into 96-well plates at a density of 8,000 cells/well (in 100 μ L). The cells were incubated at 37°C in a humidified 5% CO₂ atmosphere for 48 h to allow adherence. The culture medium was then replaced with the oral spray formulation, and the cells were incubated for different time intervals to determine the duration of cell viability. After treatment, the formulation was removed and replaced with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg/mL), followed by incubation at 37°C for 4 h. The MTT solution was then removed, and phosphate-buffered saline (pH 7.4) was used to gently wash the wells. DMSO (100 μ L) was added to dissolve the formazan crystals formed by the viable cells. The absorbance was measured at 550 nm using a microplate reader (Universal Microplate Analyzer, Packard BioScience, USA). The percentage of cell viability was calculated using the following Equation (4):

$$\text{Cell viability (\%)} = (A_{\text{sample}} / A_{\text{control}}) \times 100 \quad (4)$$

where A_{sample} is the absorbance of the treated cells, and A_{control} is the absorbance of the untreated control cells (considered 100% viability) (Fotakis & Timbrell, 2006).

2.7 Microbial contamination

Microbial contamination of the oral spray formulation (M4 formulation without preservative) was evaluated following the guidelines described in the United States Pharmacopeia 43 (USP 43). The total aerobic microbial count and total combined yeast and mold count were assessed using the procedures outlined in USP 61. Furthermore, the presence of specific objectionable microorganisms, including

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Candida albicans*, and *Clostridium* spp., was determined according to USP 43. These tests were conducted to ensure the microbiological safety of the formulation and compliance with pharmacopeia standards (USP, 2020).

2.8 Stability studies

The stability of the oral spray formulation was evaluated for up to 6 months under accelerated conditions (40°C, 75% relative humidity [RH]) and compared with storage under normal conditions (30°C, 60% RH). The SBP and MP extract contents were quantified at 1, 3, and 6 months.

2.9 Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean $\pm$ standard deviation. Statistical analysis was conducted using Student's t-test on Microsoft Excel 2019 to compare differences between formulations. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 SBP and MP extraction

After extraction, the crude extract was collected and the yield was calculated. The yields of the SBP and MP extracts were 11.45% and 25.29%, respectively (Table 2). The MP extract had a higher α -mangostin concentration ($20.61\% \pm 0.21\%$ w/w) than the SBP extract ($6.05\% \pm 0.34\%$ w/w), representing an approximately 3.4 times difference. This finding highlights the mangosteen pericarp as a more abundant α -mangostin source while also confirming the presence of this compound in propolis.

Antioxidant activity, as assessed using the DPPH assay, revealed that the MP extract had an IC₅₀ value of 35.68 ± 2.02 μ g/mL, while the SBP extract had an IC₅₀ of 63.03 ± 4.22 μ g/mL; thus, the antioxidant potency of the MP extract is approximately 1.77 times greater than that of the SBP extract. These findings are also consistent with previous studies indicating that MP extract has strong antioxidant properties that stem from its high content of xanthenes and phenolic compounds (Kosem et al., 2007). Similarly, SBP exhibits antioxidant activity attributed to its rich composition of flavonoids and phenolic acids (Vongsak et al., 2015).

The antimicrobial activity of SBP extract dissolved in DMSO against *S. pyogenes* demonstrated an MIC of 5 μ g/mL and a minimum bactericidal concentration (MBC) of 10 μ g/mL (Table 3). Similarly, MP extract dissolved in DMSO exhibited an MIC of 5 μ g/mL and MBC of 10 μ g/mL, indicating comparable antibacterial efficacy between the two extracts. These findings align with previous studies highlighting the antimicrobial potential of SBP and MP extracts, which are attributed to their rich content of flavonoids, phenolic acids, and xanthenes (Al-Hatamleh et al., 2020; Cabezas-Mera et al., 2024). Further studies are recommended to elucidate the specific components of both extracts that contribute to their antimicrobial activity against *S. pyogenes*.

Table 2. Yield, α -mangostin content, and antioxidant activity (IC_{50}) of the SBP and MP extracts

Extract	Yield (%)	α -mangostin (g per 100 g of the extract)	IC_{50} (μ g/mL)
SBP	11.45 $\pm$ 1.06	6.05 $\pm$ 0.34	63.03 $\pm$ 4.22
MP	25.29 $\pm$ 2.54	20.61 $\pm$ 0.21	35.68 $\pm$ 2.02

SBP, stingless bee propolis; MP, mangosteen pericarp; IC_{50} , 50% inhibitory concentration.

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration of the SBP and MP extracts against *Staphylococcus pyogenes*

Extract	MIC (μ g/ml)	MBC (μ g/ml)
SBP	5	10
MP	5	10

SBP, stingless bee propolis; MP, mangosteen pericarp; MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration.

3.2 Characterization of the oral spray

Table 4 presents the physicochemical characteristics of the different oral spray formulations (M1–M4). The pH ranged from 6.02 to 6.54, indicating a mildly acidic nature, which may minimize oral and throat irritation. Shear viscosity, measured between 0.46 and 1.19 mPa·s at 1 s⁻¹, indicates Newtonian fluid behavior, likely due to the absence of viscosity modifiers, which would facilitate effective dispersion.

HPLC analysis revealed that the active extract content in the oral spray formulations ranged from 99.94% to 102.27% of the labeled amount, demonstrating the stability of the bioactive compounds during formulation. The antioxidant activity was 23.82% in the extract-free formulation, while formulations M2–M4 exhibited values between 62.04% and 84.62%. In the DPPH inhibition assay, the percentage of inhibition indicates the efficiency of an extract or compound in reducing the activity of DPPH free radicals. Formulation M4 exhibited the highest activity, with 84.62% inhibition, demonstrating the greatest capacity to inhibit free radicals. Generally, antioxidant activity exceeding 50% is indicative of good inhibitory activity. The α -mangostin and flavonoid contents of the SBP and MP extracts, along with the essential oils in the formulation, contributed to the antioxidant activity exhibited by the oral spray (Calado et al., 2015).

Table 4. Physicochemical characteristics of the various oral spray formulations

Formulation	pH	Shear viscosity (mPa·s)	Antioxidant activity (%)	Content (%)
M1	6.54 $\pm$ 0.11	0.46 $\pm$ 0.04	23.83 $\pm$ 4.02	N/A
M2	6.40 $\pm$ 0.08	0.77 $\pm$ 0.03	62.04 $\pm$ 1.54	102.27 $\pm$ 1.07
M3	6.32 $\pm$ 0.07	0.89 $\pm$ 0.12	76.72 $\pm$ 2.94	101.59 $\pm$ 2.57
M4	6.02 $\pm$ 0.06	1.19 $\pm$ 0.21	84.62 $\pm$ 3.98	99.94 $\pm$ 1.27

3.3 Antibacterial activity

Table 5 details the antibacterial efficacy of the oral spray formulations against *S. pyogenes*. Notably, formulations M1–M4 demonstrated complete inhibition of *S. pyogenes* growth at a 50% concentration, confirming their antimicrobial potential. This observation aligns with the established broad-spectrum antibacterial properties of α -mangostin, which is found in mangosteen pericarp extract (Chomnawang et al., 2005). The observed antibacterial activity may be attributed to the ability of α -mangostin to impede *S. pyogenes* proliferation, reinforcing previous studies highlighting its effectiveness against gram-positive bacteria. Furthermore, the inclusion of essential oils, known for their moderate antimicrobial effects, could synergistically enhance the antibacterial activity of the formulation when combined with plant-derived compounds. Consequently, the integration of plant extracts and essential oils presents a potentially viable approach for the development of natural oral care products designed to combat pathogenic microorganisms within the oral cavity (Burt, 2004).

Table 5. Antibacterial activity of the oral spray against *S. pyogenes*

Formulation	OD ₅₅₀	Colony count (CFU/mL)
M1	0.06 $\pm$ 0.00	None
M2	0.06 $\pm$ 0.00	None
M3	0.07 $\pm$ 0.00	None
M4	0.07 $\pm$ 0.00	None
Positive control	0.11 $\pm$ 0.01	None
Negative control	1.66 $\pm$ 0.13	1.12 $\times 10^5$

OD₅₅₀, optical density at 550 nm.

3.4 Cytotoxicity

Figure 1 presents the percentage of human gingival fibroblast viability following exposure to the oral spray formulations. Cytotoxicity evaluations conducted using the MTT assay at various exposure durations revealed that all formulations induced cell death at 30, 60, and 300 s. However, at a 15 s exposure, no significant cytotoxicity

was observed, indicating a correlation between reduced exposure time and decreased cytotoxicity. Formulation M1 exhibited the highest cell viability at 75.72%, while formulation M4, containing the maximum extract concentration, exhibited a viability of 52.19%. Cell viability decreased with increasing extract concentration. This finding is consistent with previous studies indicating that high MP extract concentrations can be toxic to fibroblast cells (Charernsriwilaiwat et al., 2013). Despite this lower viability, cell viability under M4 remained above the 50% threshold, generally indicating an acceptable cytotoxicity rate, as demonstrated in previous *in vitro* studies (Mosmann, 1983). The time-dependent cytotoxicity is consistent with a previous report indicating that certain plant-derived compounds, while demonstrating antibacterial or antioxidant benefits, can also exhibit cytotoxic properties at elevated concentrations or prolonged exposure periods (Coelho et al., 2020). In practical oral applications, salivary secretions and other oral fluids may mitigate the potential toxicity of the formulation by creating a protective barrier for mucosal cells.

3.5 Microbial contamination

Table 6 presents the microbial contamination assessment of the M4 formulation without preservatives, confirming its adherence to the microbial limit specifications detailed in USP 43 (USP, 2020). The total aerobic microbial count was 6 CFU/mL, which is well within the acceptable threshold of 100 CFU/mL. Similarly, the total yeast and mold count was below detectable levels (<1 CFU/mL), satisfying the 10 CFU/mL limit. Moreover, analyses revealed the absence of *S. aureus*, *P. aeruginosa*, *C. albicans*, and *Clostridium* spp. in the 1-mL sample. Several elements contribute to the formulation's adherence to microbial safety benchmarks. The inherent antimicrobial characteristics of the plant-derived extracts and essential oils used in the composition play a significant role. These outcomes underscore the microbiological integrity of the oral spray formulation and its potential safety for various consumer applications (Herman et al., 2013).

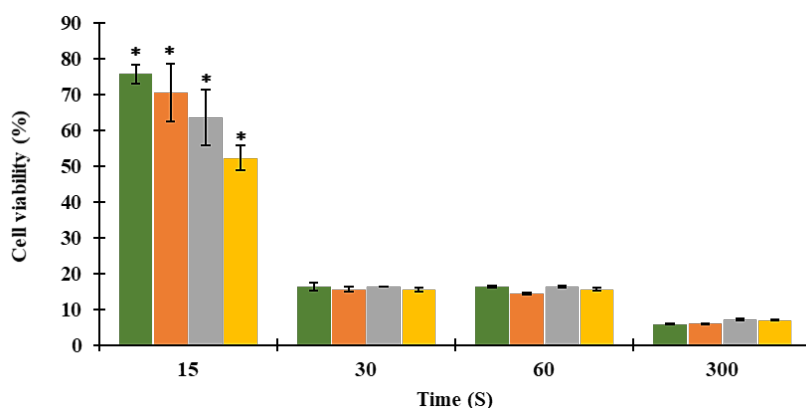


Figure 1. Cell viability of human gingival fibroblasts exposed to the oral spray formulations at various time points. M1 (■), M2 (■), M3 (■), and M4 (■). * $p < 0.05$, compared with 300 s.

Table 6. Microbial contamination test of the oral spray M4 formulation without preservatives

Test item	Criteria	Test result
Total aerobic microbial count	Not exceeding 100 CFU/mL	6 CFU/mL
Total combined yeast and mold count	Not exceeding 10 CFU/mL	<1 CFU/mL
<i>S. aureus</i>	Not detected in 1 mL	Not detected
<i>P. aeruginosa</i>	Not detected in 1 mL	Not detected
<i>C. albicans</i>	Not detected in 1 mL	Not detected
<i>Clostridium</i> spp.	Not detected in 1 mL	Not detected

3.6 Stability studies

Figure 2 illustrates the quantification by HPLC analysis of the active compounds of the oral spray formulations. Under both standard and accelerated storage conditions, the formulations exhibited consistent label claim percentages, ranging from 98.76% to 106.32%, at both the first and third months. However, by the sixth month, a significant decline in the active compound content was observed across all formulations, with percentages falling between 96.17% and 98.62%. Nevertheless, these values remained within the established acceptable range of 95%–105%, confirming the formulations' adequate stability (ICH, 2022; Praserttrungpanich et al., 2017). The accelerated stability testing suggests a potential shelf life

of approximately 2 years, which is comparable to similar commercially available products. However, further long-term stability studies are needed to establish a more precise expiration date.

4. CONCLUSION

The oral spray formulations demonstrated promising antioxidant and antibacterial properties, with α -mangostin contributing significantly to their bioactivity. Formulations M1–M4 effectively inhibited *S. pyogenes* and exhibited acceptable cytotoxicity profiles, particularly at short exposure durations. Analysis of the physicochemical

properties indicated their suitability for oral use, while microbial contamination testing confirmed their microbiological safety. Furthermore, stability studies showed that the active compounds remained within acceptable limits for up to 6 months under both normal and accelerated conditions. These findings demonstrate that oral

sprays based on SBP and MP extract are natural, safe, and effective products for oral health care applications. Future studies should expand upon these findings and explore the effects of the extracts on a wider variety of bacteria. Furthermore, clinical trials are warranted to validate the potential of these oral sprays for therapeutic applications.

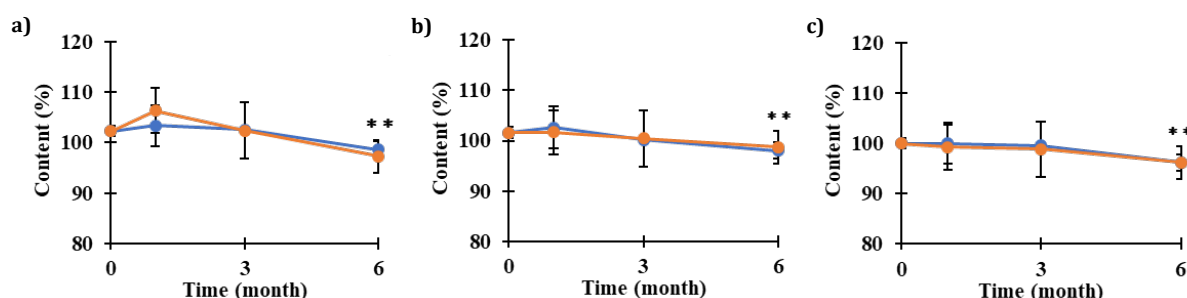


Figure 2. Content percentages of the different oral spray formulations stored under normal (30°C, 60% relative humidity [RH] ●) and accelerated conditions (40°C, 75% RH ●) at various time points. a) M2, b) M3, and c) M4. * $p < 0.05$, compared with month 0.

ACKNOWLEDGMENT

This work was financially supported by the Research Grant of the Faculty of Pharmaceutical Sciences, Burapha University (Grant No. 1/2565 (adjunct)).

AUTHOR CONTRIBUTIONS

N. C. conceived and planned the experiments. K.W., K.W., and R.T. carried out the experiment. N.C. wrote the manuscript with support from T.S. All authors discussed the results and contributed to the final manuscript.

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