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Development and evaluation of a triphala-based throat spray: Physicochemical properties, stability, and pharmacological potency

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ABSTRACT

Throat sprays are widely used, act quickly, and can help relieve a sore throat from several viral or bacterial infection. There are many types of throat sprays available today, depending on different components such as chemical compounds and herbal extracts. Triphala, a herb, consists of three dried fruits: *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica*. The chemical compounds in Triphala mainly consist of gallic acid, polyphenol, and tannin. These compounds have been reported to exhibit high antioxidant activity and anti-inflammatory effects. Then, this study aimed to develop a throat spray containing Triphala extract with suitable physicochemical properties, pharmacological potency and stability. Throat sprays with Triphala extract were investigated for their physicochemical (physical characteristics, pH, spray ability, and viscosity) and pharmacological properties (antioxidant and anti-inflammatory activity). All formulations exhibited suitable sprayability, with pH values ranging from 3.2 to 4.2 and no significant differences observed. The formulations showed significantly higher anti-inflammatory and antioxidant activities than the control. A stability study was performed over five weeks under three storage conditions. The formula containing 0.25% v/v Triphala extract exhibited suitable physicochemical and pharmacological properties. Moreover, the 0.25% v/v Triphala extract throat spray showed no precipitation under the three storage conditions, and the anti-inflammatory and antioxidant activities did not change significantly after storage. This study demonstrated a novel, suitable Triphala extract throat spray formulation as an alternative treatment for sore throat.

Keywords: throat spray; Triphala; antioxidant activity; anti-inflammatory activity

1. INTRODUCTION

The COVID-19 pandemic significantly impacted global health, presenting various symptoms that complicate the clinical management of infected patients (Mallah et al., 2021). Common symptoms include fever, cough, sore throat, nasal congestion, fatigue, loss of taste and smell, and diarrhea. Current treatments are symptomatic. Among these, sore throat is commonly reported, driving a high demand for effective symptomatic relief (Struyf et al.,

2022). Medically known as pharyngitis, a sore throat is a common and discomforting condition characterized by irritation, pain, or itchiness in the throat. It can result from various causes, including viral infections such as coronavirus, bacterial infections, or environmental factors such as dry air or pollutants. The condition is typically marked by difficulty swallowing, swollen glands, and inflamed tonsils, often accompanied by other respiratory symptoms (Lovato et al., 2020). Sore throats can be acute, lasting only a few days, or part of a more chronic condition, depending on the underlying cause. Treatment typically focuses on symptom relief and addressing the root cause, with remedies ranging from over-the-counter medications and throat sprays to more natural methods such as warm saltwater gargles (Addey & Shephard, 2012). Throat sprays have emerged as a preferred choice due to their direct application, rapid action, and safety. Given the diverse range of ingredients used, from pharmaceutical drugs (antiseptic or anesthetic agents) to herbal extracts, there is a pressing need to assess the efficacy and safety of these products. Herbal extracts, in particular, offer a promising alternative due to their potential therapeutic properties and minimal side effects (Boseila et al., 2024).

The integration of traditional herbal remedies into modern medical practice is gaining acceptance worldwide due to their natural origins and comprehensive therapeutic benefits. Among these, Triphala, a cornerstone of traditional Thai herbal medicine that has been used for a long time, has garnered attention for its potent pharmacological effects. Comprising three distinct herbal components, *Phyllanthus emblica* (Amla), *Terminalia bellirica* (Bibhitaki), and *Terminalia chebula* (Haritaki), this formulation leverages the synergistic effects of its constituents to offer robust antioxidant and anti-inflammatory properties (Ghimire et al., 2023; Peterson et al., 2017). Triphala extracts are rich in important compounds such as gallic acid, polyphenols, tannins, and flavonoids, which contribute to its healing properties. These components are known for their health-promoting properties, contributing to Triphala's efficacy in preventing oxidative stress and inflammation. The antioxidant activity stems from the ability of compounds to scavenge free radicals, reduce oxidative stress, and enhance the antioxidant defenses of the body. Similarly, the anti-inflammatory effects are attributed to inhibiting inflammatory enzymes and cytokines, providing relief and protective benefits against chronic inflammation. This rich phytochemical composition makes Triphala a potent natural remedy with broad therapeutic applications, especially in enhancing immune response and managing or preventing conditions associated with oxidative stress and inflammation (Prananda et al., 2023). Triphala is available in forms such as lozenges, tablets, capsules, and infusions, and is included in the National List of Essential Medicines of 2021. It is indicated for relieving coughs and expelling phlegm (Ghimire et al., 2023). Additionally, Triphala has been found to reduce inflammatory substances such as interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α) at wound sites (Kalaiselvan & Rasool, 2016). In the initial stages of wound inflammation, neutrophils generate free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can slow the healing process. Therefore, the antioxidants from Triphala help accelerate wound healing (Prasad & Srivastava, 2020).

In recent years, the demand for localized and rapid-acting medical treatments has increased, particularly in the management of sore throats, pharyngitis, and other throat infections (Cots et al., 2015). Throat sprays are a form of liquid medication, either a solution or suspension, that is administered as an aerosol. This method involves releasing aerosols through a nozzle using a container that does not rely on pressure to create the aerosol (Cheng, 2014). Throat sprays deliver medication directly to the affected area, ensuring quick relief and targeted action. The convenience of throat sprays allows users to apply the treatment directly to the affected area, ensuring that the active ingredients are delivered efficiently where needed most. This targeted approach not only helps reduce discomfort but also aids in the faster recovery of throat tissues (Sugibayashi et al., 2020).

Currently, there is limited research on the pharmacological potency of Triphala in the form of a throat spray. Therefore, this study focuses on developing a throat spray incorporating Triphala extract for its antioxidant and anti-inflammatory properties. This paper details the formulation process of the throat spray, evaluates its physicochemical properties and stability, and discusses its therapeutic potential in treating symptoms related to throat inflammation.

2. MATERIALS AND METHODS

2.1 Materials

Triphala extract (TPE) was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (Phra Nakhon Si Ayutthaya, Thailand). Sodium saccharin (SS), 70% sorbitol solution, xylitol, eucalyptus oil and peppermint oil were purchased from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Glycerine, menthol, methyl paraben (MP) and proply paraben (PP) were purchased from Namsiang Co., Ltd. (Bangkok, Thailand). L (+)-Ascorbic acid was purchased from Riedel-de Haën® (Seelze, Germany). Diclofenac sodium (DC) and 2,2-diphenyl-1-picrythydrazyl (DPPH) were purchased from Sigma-Aldrich (Missouri, United States). Other chemicals and reagents were of analytical grade.

2.2 Preparation of throat spray solution

To prepare the throat spray solution, each ingredient listed in Table 1 was accurately weighed. Initially, sweetening agents such as SS, 70% sorbitol solution, and xylitol were dissolved in water. Glycerin was then added and mixed thoroughly. Subsequently, menthol was incorporated until a clear solution was obtained. Flavoring agents such as menthol, eucalyptus, and peppermint oils were added. Finally, preservatives (MP:PP in a 10:1 ratio) were used to inhibit microbial growth, and the volume was adjusted with water to 100 mL. The freshly prepared throat spray solution was immediately evaluated for its physicochemical properties. The clear solution at an appropriate pH was selected to be loaded with TPE.

Table 1. The ingredients of throat spray solution formulations

Formulations	Throat spray solution ingredients (% w/v)								
	SS	Sorbitol	Xylitol	Menthol	Glycerine	Peppermint oil	Eucalyptus oil	MP : PP (10:1)	Water q.s. to
RxTS1	-	20	-	0.3	10	0.1	-	0.1	100
RxTS2	0.5	-	-	0.3	10	0.1	-	0.1	100
RxTS3	-	-	35	0.3	10	0.1	-	0.1	100
RxTS4	-	20	-	0.1	10	0.1	-	0.1	100
RxTS5	-	10	-	0.3	20	0.1	-	0.1	100
RxTS6	-	30	-	0.06	10	0.1	-	0.1	100
RxTS7	-	30	-	0.04	10	0.1	-	0.1	100
RxTS8	0.5	-	-	0.03	10	0.1	-	0.1	100
RxTS9	-	-	35	0.03	10	0.1	-	0.1	100
RxTS10	-	30	-	0.03	10	0.1	-	0.1	100
RxTS11	-	30	-	-	10	-	0.1	0.1	100
RxTS12	0.5	-	-	-	10	-	0.1	0.1	100
RxTS13	-	-	20	-	10	-	0.1	0.1	100
RxTS14	0.25	-	-	-	10	-	0.1	0.1	100
RxTS15	0.25	-	-	-	5	-	0.1	0.1	100

2.3 Preparation and characteristics of TPE-based throat spray

The throat spray solution formulations with suitable physical appearance and pH from the previous experiment (RxTS15) were selected to be loaded with 0.25%, 0.5%, and 1% TPE. These concentrations were confirmed to be the minimum needed for treatment and avoided levels that could lead to toxicity (Intatham et al., 2024; Mukherjee et al., 2022). These TPE-based throat spray formulations were evaluated for their physical appearance to check for any incompatibility and precipitation visually, and for a pH appropriate for use in the oral cavity, measured using a Horiba LAQUAtwin pH meter (Kyoto, Japan). The TPE-based throat spray was compared with the control (RxTS15), and any formulations exhibiting unstable physical appearance or pH were excluded.

2.4 Viscosity of TPE-based throat spray

The viscosity of the TPE-based throat spray was measured using a viscometer (Brookfield DV2T; Toronto, Canada) to evaluate the viscosity, ensuring it was compatible for spraying from the nozzle and application in the oral cavity. Briefly, 10 mL of the formulation was loaded into a cup, and then the rotating bob was set to rotate at an appropriate shear rate (s^{-1}) until the percentage of torque reached 80–100% at 25°C. The RxTS15 was used as a blank. The viscosity was recorded in mPa-s for each sample (Suriyaamporn et al., 2023).

2.5 Spray ability of TPE-based throat spray

The evaluation of the spraying ability of the TPE-based throat spray formulations was conducted by spraying onto moistened Kimwipes paper (Kimtech® Science™, Texas, USA), adapting the method from a previous study by Sugibayashi et al. (2020). Briefly, the TPE-based throat spray formulation was sprayed once onto paper from a distance of 3 cm. Each push of the spray delivered approximately 6 µL. The spray area, where the brown color from TPE was visible, was then measured for diameter.

2.6 Antioxidant activity of TPE-based throat spray

The antioxidant activity of a TPE-based throat spray was assessed using the DPPH free radical scavenging activity assay, following a previous study by Samprasit et al. (2024). In brief, 100 µL of freshly prepared 0.2 mM DPPH solution in methanol was added to each TPE-based throat spray at different concentrations at the same volume in a 96-well plate. The mixture was then vigorously agitated and incubated in a dark incubator at 37°C for 30 min. Ascorbic acid (AA) at 0.001% w/w was used as the positive control. Subsequently, the percentage of antioxidant activity was measured using a microplate reader at an absorbance of 515 nm and calculated according to Equation 1.

$$\% \text{ Antioxidant activity} = (A_{control} - A_{test})/A_{control} \times 100 \quad (1)$$

where A_{test} is the absorbance of the DPPH solution after reacting with the sample and $A_{control}$ is the absorbance of the DPPH solution without the sample.

2.7 Anti-inflammation of TPE-based throat spray

The anti-inflammation effect of the TPE-based throat spray was tested using the protein denaturation inhibition method, following a previous study by Sofiane et al. (2015). Briefly, 0.2 mL of egg albumin (from a fresh hen's egg) was mixed with 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) as a protein mixture. Then, 2 mL of various concentrations of the TPE-based throat spray was added to the protein mixture in equal volumes in a 96-well plate. A similar volume of double-distilled water served as the control. Different concentrations of diclofenac sodium were used as the reference standard. Subsequently, the samples were incubated at 37±2°C for 15 min and then heated at 70°C for 5 min. After cooling, the percentage of protein denaturation inhibition by the TPE-based throat spray and the standard was measured using a microplate reader at an absorbance of 660 nm and calculated according to Equation 2.

$$\% \text{ Protein denaturation inhibition} = (M_{control} - M_{test})/M_{control} \times 100 \quad (2)$$

where M_{test} is the absorbance of a protein mixture after reacting with the sample and $M_{control}$ is the absorbance of a protein mixture without the sample.

2.8 Stability of TPE-based throat spray

The stability test of TPE-based throat spray was kept in a tight container and stored at 5±3°C, and 25±2°C/60±5% RH for five weeks, following the ICH Q1A(R2) guidelines. To assess the stability of light exposure, the TPE-based throat spray was stored in a light-protected container and a clear bottle at room temperature for 5 weeks to compare physical stability. After completing the stability tests, physical appearance, pH, viscosity, spray ability, and antioxidant properties of each formulation were evaluated (Suriyaamporn et al., 2024).

2.9 Statistical analyses

All experiments were conducted in triplicate. The results were expressed as the mean ± standard deviation. Data were statistically analyzed using one-way ANOVA and Tukey's HSD post-hoc test for multiple group comparisons. For comparisons between two groups, the independent t-test was employed. Statistical significance was set at a *p*-value of less than 0.05, Statistical analyses were performed using Microsoft Excel 2021 with the data analysis extension toolbar.

3. RESULTS

3.1 Characteristics of throat spray solution and TPE-based throat spray

The physical appearance of formulations RxTS1 to RxTS3 was assessed to select the sweetening agent for the throat spray solution. The results showed that SS, sorbitol, and xylitol exhibited suitable physical stability without phase separation. However, when menthol was added to the solution, it did not dissolve in any of the formulations, RxTS1 to RxTS10. Consequently, menthol and peppermint oil were replaced with eucalyptus oil in formulations RxTS11 to RxTS15. SS was chosen as the sweetening agent because it was universally accepted and safe for use in the oral cavity. In formulation RxTS15, reducing the amounts of SS and glycerin led to a change in the throat spray formulation pH to approximately 3.93±0.12, which is suitable for the oral cavity.

Subsequently, the optimal formulation was loaded with TPE at various concentrations. The results showed that the physical appearance of the TPE-based throat spray exhibited a slight increase in brown color with increasing concentrations of TPE. The 1% of TPE-based throat spray showed slight sediment in the formulation. The pH of the TPE-based throat spray over the concentrations ranged from 3.50±0.12 to 3.70±0.02. This result reported no significant differences among the three formulations. Figure 1 illustrates the physical appearance of the throat spray solution as the control (RxTS15) and TPE-based throat sprays at various concentrations from 0.25% to 1%.

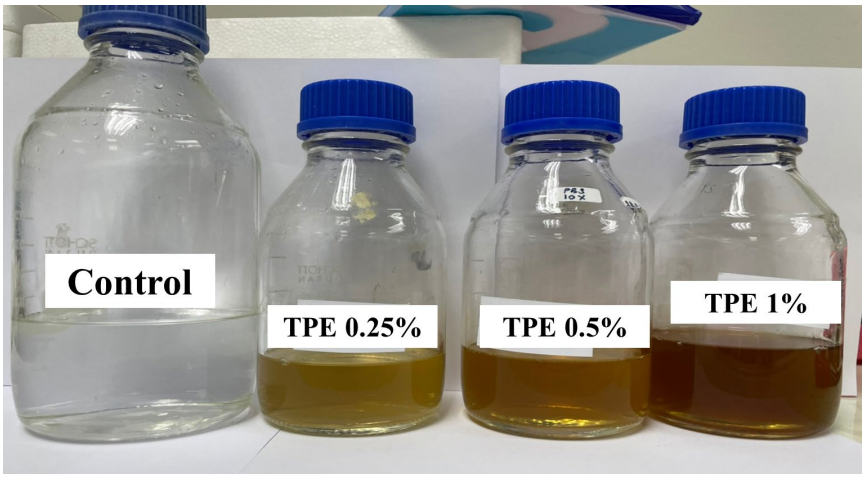

Figure 1. Physical appearance of throat spray solution: Control (RxTS15) and TPE-based throat spray at various concentration from 0.25% to 1%

3.2 Viscosity of TPE-based throat spray

The viscosity of the throat spray solution, before and after loading TPE, showed no significant differences in every concentration. However, there was a slight decrease in viscosity after TPE was loaded into the throat spray solution. The viscosity of the throat spray solution was 4.2±0.3 mPa-s. Furthermore, the viscosities of the throat spray solutions loaded with TPE at concentrations of 0.25%, 0.5%, and 1% are reported in Table 2.

3.3 Spray ability of TPE-based throat spray

The spray ability of the throat spray solution before and after loading TPE was easy to manage from a spray bottle and did not obstruct the nozzle in any formulation. The diameter of the solution sprayed widely on Kimwipes paper as approximately in the range of 6.10±0.125 cm to 7.00±0.17 cm. The diameter after spraying in each formulation showed no significant difference compared to the control. The diameter results of spray ability are reported in Table 2.

3.4 Antioxidant activity of TPE-based throat spray

The antioxidant activity of TPE-based throat spray at different concentrations was compared with an ascorbic acid solution, as shown in Figure 2. It was found that there was no significant difference in the antioxidant activity of TPE when the concentration was increased. Moreover, every

TPE concentration in the throat spray solution showed no significant difference in antioxidant activities compared to the standard 0.001% w/v ascorbic acid solution (94.68±3.70%). However, only the throat spray solution showed no antioxidant activity (less than 80%). The antioxidant activity of the TPE-based throat spray is detailed in Table 2.

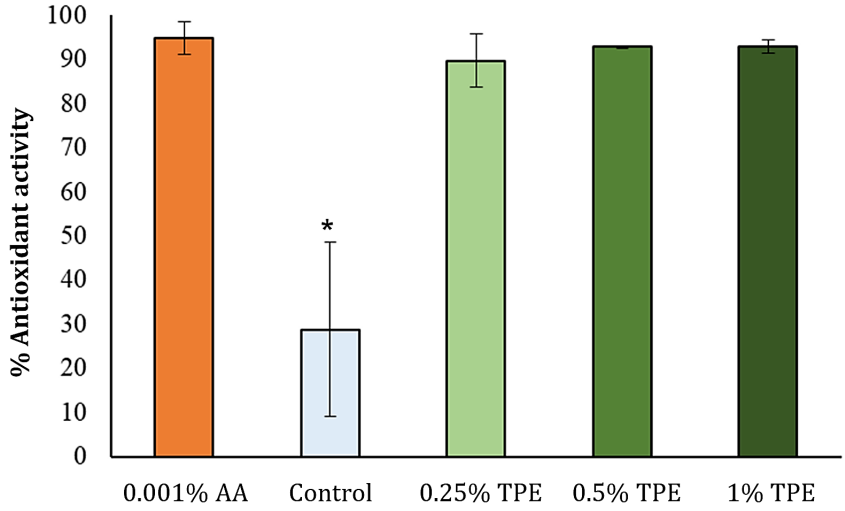

Figure 2. Antioxidant activity of TPE-based throat spray compared with a control and ascorbic acid (AA)
Note: *indicate significant lower than others

3.5 Anti-inflammation of TPE-based throat spray

The *in vitro* anti-inflammatory test for the TPE-based throat spray demonstrated protein denaturation inhibition as represented in Figure 3. The throat spray solution base (water) showed a slight anti-inflammatory effect, significantly lower than that of every TPE-based formulation. For the positive control, diclofenac exhibited a significantly higher anti-inflammatory effect at concentrations greater than 0.04% when compared with every concentration of TPE. The results conclude that the anti-inflammatory properties of TPE at every tested concentration were equivalent to those of diclofenac at a dose of 0.04%.

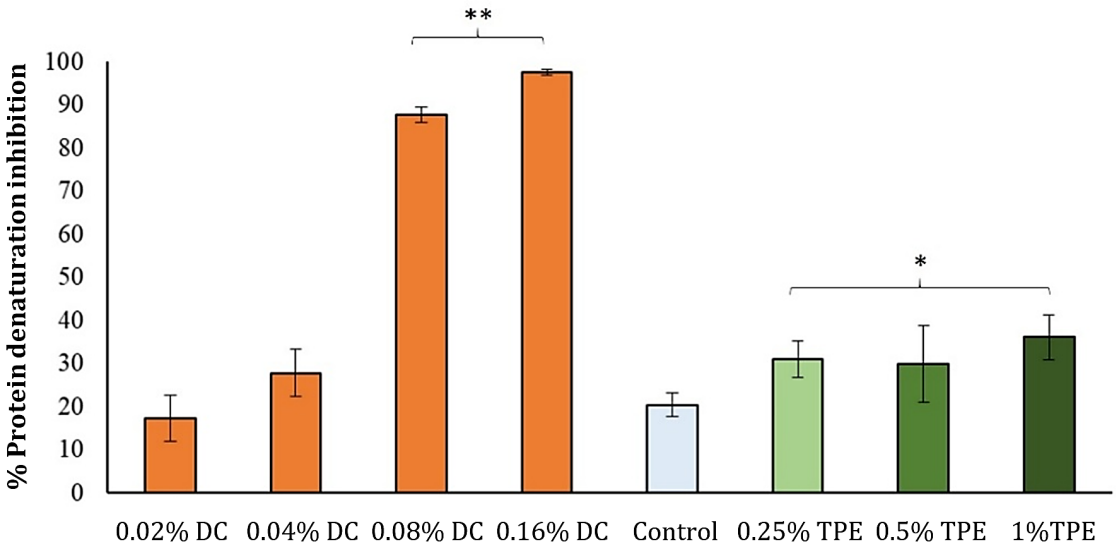

Figure 3. Protein denaturation inhibition compared with a control and the difference concentration of diclofenac sodium (DC)
Note: *indicates significant difference higher than control, **indicate significant higher than every concentration of TPE-based throat spray

3.6 Stability of TPE-based throat spray

After five weeks of stability testing, the TPE-based throat spray formulations showed stable physical properties in the control, 0.25%, and 0.5% concentrations. However, the 1% TPE-based throat spray exhibited heavy sedimentation under stability conditions at 25°C, both with and without light exposure. Additionally, the pH, viscosity, spray ability, and antioxidant properties of the control and TPE-based throat sprays at all concentrations did not change significantly compared to the results from week zero during the stability testing. The stability results of TPE-based throat spray after five weeks under different conditions are reported in Table 2.

Table 2. Stability of TPE-based throat spray after 5 weeks with difference conditions

Formulation	Physical appearance	pH	Viscosity (mPa.s)	Spray ability (cm)	Antioxidant activity (%)
Stability condition: 0 week					
Control	Clear	3.93±0.12	4.20±0.30	6.50±0.44	28.79±19.79%
0.25% TPE-based throat spray	Light brown	3.70±0.02	3.70±0.17	7.00±0.70	89.62±6.03%
0.5% TPE-based throat spray	Brown	3.60±0.03	3.80±0.35	6.57±1.01	92.73±0.06%
1% TPE-based throat spray	Dark brown with slightly sediment*	3.50±0.12	3.20±0.69	6.10±1.25	92.93±1.55%
Stability condition: 4°C					
Control	Clear	4.00±0.01	3.90±0.01	6.93±0.91	20.90±7.66%
0.25% TPE-based throat spray	Light brown	3.60±0.01	4.01±0.17	6.17±1.27	93.83±0.64%
0.5% TPE-based throat spray	Brown	3.50±0.02	3.80±0.17	6.13±0.47	93.03±0.45%
1% TPE-based throat spray	Dark brown with slightly sediment*	3.43±0.06	4.10±0.35	5.10±0.10	95.51±1.33%
Stability condition: 25°C with light exposure					
Control	Clear	3.83±0.06	3.60±0.01	5.90±0.62	20.46±2.71%
0.25% TPE-based throat spray	Light brown	3.43±0.06	3.90±0.01	6.07±0.23	93.16±0.03%
0.5% TPE-based throat spray	Brown	3.50±0.01	3.80±0.17	6.07±0.23	92.61±0.57%
1% TPE-based throat spray	Dark brown with heavy sediment*	3.30±0.01	4.30±0.17	6.00±0.23	93.88±0.73%
Stability condition: 25°C with light protection					
Control	Clear	3.83±0.06	3.70±0.35	5.83±0.91	21.46±2.21%
0.25% TPE-based throat spray	Light brown	3.50±0.01	3.70±0.17	5.60±0.36	94.11±0.31%
0.5% TPE-based throat spray	Brown	3.30±0.01	4.00±0.17	6.27±1.42	93.87±0.73%
1% TPE-based throat spray	Dark brown with heavy sediment*	3.20±0.03	4.20±0.01	5.40±0.85	93.95±1.59%

Note: *Unstable formulation, excluded for next study.

4. DISCUSSION

From the throat spray solution development results, SS, sorbitol, and xylitol showed suitable physical stability without any phase separation. These three sweetening agents are widely used in various food and drug products (Aronson, 2016; Nirmala et al., 2022). SS has an intensely sweet flavor, about 300 to 500 times sweeter than sucrose. However, it often has a bitter or metallic aftertaste, especially when used in high concentrations (Jolly, 2004). Sorbitol and xylitol are less sweet than sucrose, producing a noticeable cooling effect when dissolved in the mouth. Moreover, as sugar alcohols, sorbitol and xylitol can lead to digestive issues such as gas, bloating, and diarrhea, particularly in individuals with sensitive digestive systems (Msomi et al., 2021). Therefore, 0.25% SS was selected as the sweetening agent in the throat spray solution to achieve the desired taste without a bitter or metallic aftertaste. Menthol and peppermint oil were added to the throat spray solution to provide a cooling effect in the mouth; however, they did not dissolve in any of the formulations due to their chemical structure and nature. Both substances are classified as organic compounds that are fundamentally hydrophobic, meaning they do not interact well with water molecules, which are polar (Patel et al., 2007). Thus, menthol and peppermint oil were removed and replaced with eucalyptus oil in the formulations. Therefore, the RxTS15 was the optimal base formulation to mix with TPE.

After mixing with TPE, the results revealed that the physical appearance of the TPE-based throat spray at concentrations of 0.25% and 0.5% was light brown and brown, respectively, with no particles or sediment. However, increasing the concentration of TPE to 1% resulted in the formation of a brown sediment in the formulation. These results may suggest that the TPE exceeded its solubility in the throat spray solution (RxTS15) (Ashokkumar, 2007). The pH of the throat spray formulations, with and without TPE, showed no significant differences, indicating that TPE did not alter the pH at any concentration. The throat spray formulations were mildly acidic (pH 3 to 4), which was still considered safe and comfortable for the oral and throat cavity. The Triphala throat spray, formulated without the addition of sodium saccharin as a sweetener and glycerin as a humectant, tended to be more acidic due to the Triphala extract. Triphala extract has slightly acidic properties, with a pH range of approximately 3.5 to 5.0 (Panomsuk et al., 2021; Sharma et al., 2014). This shift in pH can be attributed to the absence of sodium saccharin, which slightly leans towards being basic, and glycerin, which could interact with other components to stabilize the pH. Without these ingredients, the relative concentrations and effects of any acidic components in the spray became more pronounced, leading to a lower pH. This reduction in components also meant the spray had a reduced capacity to neutralize any acidic variations, further contributing to its overall acidity (Panomsuk et al., 2021). However, oral care products should typically be formulated closer to neutral (pH between 6.2 and 7.6) (Baliga et al., 2013; Pelvan et al., 2022) to avoid irritation. The throat spray formulation should be improved in future studies, allowing the pH to approach neutrality.

All formulations showed no significant differences in the viscosity and spray ability results. The TPE concentrations used in throat spray (0.25%, 0.5%, and 1%) did not significantly increase the viscosity. This is because the differences between these concentrations were minimal,

only increasing from 0.25% to 0.5%. Therefore, when combined into the complete formulation totaling 100 ml, these variations did not lead to noticeable differences in viscosity among the formulations. The spray ability of formulations generally depends on the viscosity of the formulation; that is, if the viscosity is high, the nozzle of the spray vial is easily blocked by the formulation. When the concentration of TPE was increased, the viscosity did not increase, making it easy to spray from the nozzle. The appropriate viscosity for throat spray formulations should not exceed approximately 14.0 mPa·s (Sugibayashi et al., 2020; Umar et al., 2020). Moreover, the diameter of the spray in every formulation was suitable for use in the oral cavity when compared with previous studies (approximately 2-8 cm). If the diameter is too small, the spray cannot reach all areas in the oral cavity; meanwhile, if it is too large, it cannot reach the deeper sites (the throat cavity) (Sugibayashi et al., 2020).

In antioxidant activity, every concentration of TPE-based throat spray exhibited strong antioxidant activity compared to the ascorbic acid standard (% antioxidant more than 80%). Meanwhile, the control or RxTS15 without TPE had no antioxidant activity. The natural recovery process of such ulcers unfolds in distinct phases, starting with an inflammation stage marked by the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Antioxidants are essential during wound healing as they help mitigate oxidative stress. Additionally, research indicates that Triphala extract demonstrates significant antioxidant properties, effectively scavenging free radicals at concentrations exceeding 10 µg/mL or 0.001% w/v (Babu et al., 2013). Given these findings, the 0.25% TPE-based throat spray was effective enough to manage sore throat or pharyngitis.

Denaturation of tissue proteins is a recognized factor in inflammation. When proteins in the throat are denatured, they can reveal new antigens, potentially initiating a Type III hypersensitivity reaction (Sofiane et al., 2015; Umapathy et al., 2010). This process forms immune complexes that deposit in the throat tissues, causing inflammation and symptoms such as a sore throat or pharyngitis. These reactions typically occur in response to infections or allergic reactions that alter protein structures, leading to an immune response against these newly exposed antigenic sites. Additionally, studies have shown that heat-denatured proteins can provoke delayed hypersensitivity as effectively as their native counterparts (Sofiane et al., 2015). Research also indicates that conventional NSAIDs like diclofenac act not only by inhibiting the production of endogenous prostaglandins through COX enzyme blockade but also by preventing protein denaturation (Gunaydin & Bilge, 2018; Rouzer & Marnett, 2020). Comparatively, a TPE-based throat spray demonstrated weaker anti-inflammatory effects than diclofenac at concentrations exceeding 0.04% w/v (Jantrapirom et al., 2021). Tannins and polyphenols in the TPE-based throat spray enhanced anti-inflammatory action primarily through their antioxidant properties. Tannins form a protective barrier on inflamed tissues, reducing irritation, while polyphenols neutralize damaging free radicals and reactive oxygen species, which are key in inflammatory conditions. They also inhibited inflammatory mediators like cyclooxygenase enzymes, decreasing prostaglandin synthesis. These compounds modulated critical inflammatory pathways, including inhibiting NF-kB, reducing cytokine production, and influencing cell survival. This dual action made them effective in managing inflammation in sore throat treatments (Prananda et al., 2023).

The stability study of all formulations demonstrated that pH, viscosity, spray ability, and antioxidant activity showed no significant changes from the initial conditions. However, the physical appearance of the 1% TPE-based throat spray exhibited heavy sedimentation under stability conditions at 25°C, both with and without light exposure. This suggests that storage temperature may affect TPE solubility (Ashokkumar, 2007). In contrast, lower concentrations of TPE did not result in any sedimentation. Therefore, the 0.25% TPE-based throat spray proved to be the most suitable formulation in this study, offering the desired physical appearance, pH, viscosity, and spray ability. Additionally, it provided high antioxidant and anti-inflammatory effects at the lowest concentration of TPE.

5. CONCLUSION

In this study, the 0.25% TPE-based throat spray, which used SS as a sweetening agent, exhibited appropriate physical properties, pH, viscosity, spray ability, antioxidant activity, and anti-inflammatory effects, indicating its potential efficacy in treating sore throat or pharyngitis in patients. This throat spray formulation also demonstrated high stability over five weeks. Further studies are required to improve the pH of the throat spray formulation to approach neutrality. Comprehensive analysis including antimicrobial testing, cytotoxicity, and clinical tests is also necessary.

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