Anti-inflammatory, Antibacterial Properties and Tannin Contents of Thai Spice Water Extracts

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Abstract

The objective of this research was to develop water-based extracts of Thai spices, prepared using traditional decoction techniques, to assess their potential medicinal properties. These extracts provide a foundation for developing a Thai spice-based oral spray. To achieve this, the study investigated the *in vitro* anti-inflammatory activity, which was assessed using the albumin denaturation inhibition method with egg albumin, and compared that to the standard drug Diclofenac diethylammonium. The antibacterial properties against the oral pathogenic bacterium *Streptococcus mutans* were tested using the disc diffusion method and compared with the standard antibiotic Erythromycin, and total tannin content using the Folin-Ciocalteu colorimetric technique. The extraction process was enhanced through the application of the reflux method to optimize the development of the extracts. Three types of Thai spices; Chinese cinnamon (*Cinnamomum cassia* L.), clove (*Syzygium aromaticum*), and star anise (*Illicium verum*), were extracted using water as a solvent, with a ratio of 1:10 (w/v). The extraction process was conducted for two different durations, resulting in six extract samples as 1-hour extractions; Chinese cinnamon extract (E1A), clove extract (E1B), and star anise extract (E1C), and 3-hour extractions; Chinese cinnamon extract (E3A), clove extract (E3B), and star anise extract (E3C). These six extracts were tested for their *in vitro* anti-inflammatory activity, antibacterial activity against *S. mutans*, and total tannin content. The results showed that E1B exhibited the strongest *in vitro* anti-inflammatory activity, with an IC_{50} value of 1.17±0.05 mg/ml, which was significantly higher than that of the other extracts (p<0.05). Additionally, extracts obtained from 1-hour extraction demonstrated higher anti-inflammatory effects than 3-hour extraction. Furthermore, E1B contained the highest total tannin content (87.89±2.51 mg TAE/g extract). However, when tested for antibacterial activity against *S. mutans*, none of the six extracts showed any inhibitory effects compared to the standard antibiotic Erythromycin (23.23±0.17 mm). This finding is crucial for future studies exploring these Thai spices for many medicinal uses, particularly their potential as natural anti-inflammatory agents in various products."

Keywords: Thai spices extract, Anti-inflammatory activity, Total tannin content, Antibacterial activity

Introduction

The use of medicinal plants has been an integral part of traditional healing practices and product development in various civilizations for thousands of years. Thai spices, particularly Chinese cinnamon (*Cinnamomum cassia* L.), clove (*Syzygium aromaticum*), and star anise (*Illicium verum*) are economically valuable spices that continue to play a significant role in both traditional medicine and modern industries. These spices are not only high-value commodities in the global market but also serve as essential raw materials for the development of health-related products and in other industries. The increasing demand for these spices, particularly in the health and functional food industries, has driven further research into effective extraction methods for bioactive

compounds. Cinnamon, derived from *Cinnamomum zeylanicum* and *Cinnamomum cassia*, is prized not only for its therapeutic properties such as anti-inflammatory and antimicrobial effects from cinnamaldehyde, but also for its widespread use in the food, cosmetic, and pharmaceutical sectors. Similarly, star anise, rich in trans-anethole, is in high global demand for its aromatic and medicinal qualities, with particular importance as a natural source of shikimic acid, a key compound in antiviral drug production. Clove, known for its high eugenol content, is widely used in dentistry, food preservation, and health products due to its strong antimicrobial and anti-inflammatory activity. The enduring popularity and commercial relevance of these spices highlight their dual importance as traditional remedies and valuable bioactive ingredients in contemporary natural health products and global markets. (Gunawardena et al., 2015; Sabry et al., 2021; Mayekar et al., 2021)

In Thai traditional medicine, clove (*Syzygium aromaticum*) has been used to alleviate pain, reduce fever, and treat oral infections such as tooth decay and gingivitis. Research has found that eugenol, the main compound in clove, exhibits analgesic, anti-inflammatory, and antibacterial properties (Pulikottil & Nath, 2015; Taher et al., 2015; Devi et al., 2024; Damasceno et al., 2024). Chinese cinnamon (*Cinnamomum cassia* L.) contains active compounds such as cinnamaldehyde and cinnamic acid, which exhibit anti-inflammatory and antibacterial properties (Gunawardena et al., 2015; Nabavi et al., 2015). Chinese cinnamon is commonly used in the food industry as a natural preservative and in the cosmetic industry for making anti-inflammatory skin creams (Vasconcelos et al., 2018). Star anise (*Illicium verum*), which originates from China and Vietnam, is now commonly found in Thailand. It contains key compounds such as trans-anethole, linalool, and shikimic acid. These compounds have been reported to exhibit anti-inflammatory, antioxidant, and antiviral activities. Studies suggest that star anise can be used in the treatment of respiratory infections, including influenza (Nguyen et al., 2021; Majali, 2022; Sharafan et al., 2022).

Several studies have confirmed the anti-inflammatory and antibacterial properties of water extracts from cinnamon, clove, and star anise. Chinese cinnamon water extract has demonstrated significant anti-inflammatory effects, attributed to its bioactive compounds that act synergistically, making it more effective than isolated compounds alone (Khedkar & Khan, 2023). Additionally, research in diabetic animal models showed that cinnamon extract reduced oxidative stress and improved cognitive performance, supporting its anti-inflammatory potential (Mesripour et al., 2016). Clove water extract has also been shown to exhibit strong anti-inflammatory activity by significantly reducing the production of pro-inflammatory mediators such as IL-6, TNF-α, and nitric oxide in LPS-stimulated macrophages. These effects are linked to the inhibition of the NF-κB signaling pathway and related gene expression (Gurusmatika et al., 2024). For star anise, water extracts were found to contain phenolic compounds such as coumarin, apigenin, and rosmarinic acid, which contributed to its antibacterial effects against several bacterial and fungal strains (Sabry et al., 2021). While these studies did not specifically quantify tannin content, they highlight the potential of water-based extractions from these spices as sources of anti-inflammatory and antibacterial agents.

Streptococcus mutans is a Gram-positive bacterium recognized as a primary contributor to dental caries due to its ability to thrive in sugary and acidic environments, where it produces acid that erodes tooth enamel. It forms biofilms by converting sucrose into sticky polysaccharides, enabling it to firmly adhere to tooth surfaces and resist removal. While standard prevention strategies such as regular brushing, fluoride toothpaste, dietary sugar reduction, and professional dental care are widely recommended, they often depend heavily on individual compliance and daily habits. Furthermore, commonly used antibacterial agents such as chlorhexidine, although

effective, are associated with side effects such as altered taste, tooth staining, and disruption of the natural oral microbiota. These limitations highlight the need for safer, more effective and user-friendly alternatives, particularly those derived from natural sources with proven antimicrobial and biofilm-inhibiting properties (Almoudi et al., 2018; Lemos et al., 2019).

Tannins are natural polyphenols found in many plants. They have complex structures and vary in size. There are two main types: hydrolyzable tannins and condensed tannins. Hydrolyzable tannins break down into simpler compounds like gallic acid and glucose, while condensed tannins form through the combination of flavan-3-ol units. Tannins, which are phenolic compounds, that are commonly found in leaves, seeds, bark, and unripe fruits, are known for their astringent and bitter taste (Das et al., 2020), and are known for their anti-inflammatory, antioxidant, and antimicrobial properties. One of their key anti-inflammatory mechanisms is inhibiting the production of cytokines, which are proteins that play a crucial role in the body's inflammatory response. (Souza et al., 2007)

In Thai traditional medicine, one common method of preparing herbal remedies is through decoction. This process involves boiling dried or fresh herbs in water. The parts of the plant used for decoction can include leaves, stems, heartwood, seeds, and roots. The herbs are cut or chopped into suitable sizes and placed into a pot made of earthenware, ceramic, or non-metallic materials. Sufficient water to cover the herbs is added to the pot. If dried herbs are used, they should first be soaked in water as preparation for the boiling process. Fresh herbs can be used immediately without soaking. The mixture is then brought to a boil over medium heat. Once it starts boiling, the heat is lowered to a simmer, and the herbs are stirred occasionally. In Thai tradition, the decoction method follows a "3:1" rule, meaning that three parts of water are used, and the volume is reduced to one part by boiling. The boiling time depends on the plant part being used. For soft plant parts like leaves, flowers, or small twigs, 3 or 4 min is usually sufficient. However, for tougher parts like roots or heartwood, a longer boiling time of about 10 min is required to extract the medicinal properties. This method is simple yet effective in creating herbal remedies based on Thai traditional wisdom. (Phanpheng et al., 2021).

However, further research into formulation development is warranted. In this study, an extraction method inspired by Thai traditional herbal preparation techniques, specifically decoction, was adapted for laboratory use through reflux extraction. Three spices; Chinese cinnamon, clove, and star anise, were used, using drinking water as the extraction solvent. The goal was to develop a safe and environmentally friendly Thai spice extract. The intention was to be able to use the resulting extract to formulate an oral spray containing the Thai spice extracts. Optimizing Thai traditional medicine herbal extraction techniques by integrating water-based extraction with scientific principles, refining the process, and elevating its standardization were the intended outcomes of the research. The three spices selected are commonly used in Thai cuisine and remain widely popular in households. Their established safety makes them suitable for development into an extract for use in an oral spray. Since the spice extract obtained from this study is intended for development into an oral spray product with anti-inflammatory properties for the mouth and throat, as well as oral cavity infection prevention, it is essential to evaluate its biological activities. Therefore, the Thai spice extracts were tested *in vitro* for anti-inflammatory activity, total tannin content, and their inhibitory effect against *S. mutans*, an oral bacterium primarily responsible for tooth decay (Loesche, 1986). The findings from this study will provide valuable data to support the development of an oral spray containing Thai spice extracts, ensuring its credibility and enhancing its potential for effective commercialization.

Materials and Methods

Materials

Chinese cinnamon, clove, and star anise were obtained from the Lanna Herbs Shop, Chiang Mai, Thailand. Distilled water was obtained from Syndicate Co., Ltd., Thailand. Potable water was obtained from Singha Corporation Co., Ltd., Thailand. *Streptococcus mutans* (ATCC 25175) was obtained from the Thailand Institute of Scientific and Technological Research. Erythromycin was obtained from Oxide Ltd., United Kingdom. Brain Heart Infusion was obtained from HiMedia Laboratories Pvt. Ltd., India. Diclofenac diethylammonium (Difelene®) was obtained from Thailand. Tannic acid was obtained from Loba Chemie™, India. Sodium percarbonate was obtained from RCI Labscan, Thailand. Folin-Ciocalteu reagent was obtained from RCI Labscan, Thailand.

Methods

Preparation of spice raw materials

The spices used for extraction were examined for their physical characteristics based on previous studies of Thai spices. Cinnamon was referenced by Spence (2024), clove from Mayekar et al. (2021), and star anise from Tassou et al. (2012), all of which are credible sources. Additionally, the herbal medicine store supplying the raw materials for this extraction is supervised by a licensed Thai traditional medicine expert specializing in Thai pharmacy. This expert ensures the quality and authenticity of the herbs used as raw materials in herbal medicine preparation.

Dried herbal samples of the Chinese cinnamon, clove, and star anise were coarsely ground and then re-dried to prevent moisture and fungal contamination during extraction. The drying process was carried out in a hot air oven at 50°C for 1 hr. After drying, the samples were removed from the oven and left to cool at room temperature for 30 min (see **Fig.1**).

(A) (B) (C)

Figure 1 (A) Chinese cinnamon (*Cinnamomum cassia* L.), (B) Clove (*Syzygium aromaticum*), and (C) Star anise (*Illicium verum*)

Preparation of spice extractions

The extraction process was performed using the reflux extraction method, with water as the solvent. This method involves immersing the herbal samples in the solvent and applying direct heat to facilitate the dissolution of active compounds from the herbs. The ratio of herb to solvent was set at 1:10 (w/v). A 20-gram sample of each spice was weighed separately and put into a round-bottom flask and 200 ml of water was added. The flask was placed in a heating mantle and the temperature adjusted to 50±2°C. The extraction process was started after assembling the condenser, with the extraction temperature maintained at 50±2°C. The extraction was carried out for two different durations of 1 hr and 3 hr. After the designated extraction time, the extracts were filtered using filter paper with a diameter of 110 mm, separating the liquid extract from the solid residues.

The choice of 1 hr or 3 hr extraction times was made to compare the efficiency of extracting active compounds from the herbs. This approach helped to determine the duration that yielded the highest concentration of compounds. Testing the different extraction times also allowed for understanding the impact of time on the quality and quantity of the extract, and ensured that the extraction process was optimized, balancing both time and resources to avoid unnecessarily long extractions that do not improve results.

***In vitro* anti-inflammatory activity test.**

The anti-inflammatory activity of the various extracts was tested *in vitro* by evaluating their ability to inhibit heat-induced albumin denaturation in egg albumin, measured through absorbance. The anti-inflammatory effects were assessed by calculating the concentration required to inhibit 50% of albumin denaturation (IC_{50}), and the results were compared to the inhibition achieved with the standard drug Diclofenac diethylammonium. The assay was adapted from the method described by Fakir et al. (2024). The extract samples and the anti-inflammatory drug Diclofenac diethylammonium were dissolved in distilled water. The samples were centrifuged, and the supernatant was collected and diluted to final concentrations of 0.625, 1.25, 2.5, 5, and 10 mg/ml. The prepared sample solutions were then incubated with egg albumin at 70±2°C for 5 min. The absorbance was measured to determine the extent of albumin denaturation compared to the standard reference. The experiment was performed in triplicate (n=3). The IC_{50} value (the concentration required to inhibit 50% of albumin denaturation) was calculated to evaluate the anti-inflammatory potential of the extracts.

***In vitro* antibacterial activity test.**

An antibacterial activity test against *S. mutans* was done using the Disc Diffusion method in a laminar flow biohazard class II cabinet (Renovation Technology, Thailand). The assay was modified from the method described by Elrotob and Kabalci (2024). The extract samples were dissolved in distilled water and then sterilized by filtration through a 0.2 µm pore membrane. The samples were diluted using sterile techniques to achieve concentrations of 10, 100, and 1000 mg/ml, as well as an undiluted sample. The selected bacterial strain for testing was *S. mutans* ATCC 25175 (Department of Medical Sciences, Ministry of Public Health, Thailand), a bacterium known to thrive in the human oral cavity. The extract's antibacterial activity against *S. mutans* was assessed using the disc diffusion method. A volume of 10 µl of the prepared sample was applied onto a 6 mm filter paper disc to achieve concentrations of 0.1, 1, and 10 mg, as well as an undiluted sample. This was compared with the standard antibiotic, Erythromycin (0.015 mg). *S. mutans* suspension was adjusted to a 0.5 McFarland standard turbidity, then evenly spread across the surface of BHI agar using a cotton swab. Filter paper discs preloaded with extract samples were placed on the agar surface, while the solvent used to dissolve the samples served as the negative control. The inoculated plates were incubated at 37±1°C for 24 hr. Antibacterial activity was determined by measuring the diameter of the inhibition zone around each disc. The experiment was performed in triplicate (n=3).

Total tannin content test

The assay for total tannin content was adapted from the method described by Martins et al. (2020) using the Folin-Ciocalteu colorimetric technique. Tannic acid served as the standard reference. A 0.2 ml aliquot of the tannic acid standard solution or extract sample was mixed with 0.8 ml of 10% (v/v) Folin-Ciocalteu reagent. The mixture was thoroughly shaken and incubated at room temperature for 5 min. After incubation, 1.0 ml of 2.5% (w/v) sodium carbonate solution was added and thoroughly mixed. The mixture was incubated at room temperature for 20 min, and its absorbance was measured at 760 nm using a UV-Vis spectrophotometer.

The experiment was performed in triplicate (n=3). The total tannin content of the extracts was calculated using a tannic acid standard calibration curve and expressed as tannic acid equivalents (mg TAE/g extract).

The statistical analysis

The statistical analysis used in this study involved the calculation of standard deviation (SD) and pairwise comparison of means using the paired t-test (p<0.05) for the biological activity tests of the spice extracts.

Results

The characteristics of the six extract samples are as follows: 1 hr extraction; E1A was a brownish-red liquid, E1B extract was a dark brown liquid, and E1C was a yellow liquid. For the 3-hour extraction, E3A was a dark brownish-red liquid, E3B was a dark brown liquid, and E3C extract was a yellow-brown liquid. The % yields of each extraction are shown in Table 1.

The *in vitro* anti-inflammatory activity of all six extract samples was evaluated, and it was found that all the extracts exhibited anti-inflammatory effects in the test. The IC_{50} values, representing the concentration required to inhibit 50% of albumin degradation, were: E1A, 1.96±0.11 mg/ml, E1B, 1.17±0.05 mg/ml, E1C, 2.42±0.06 mg/ml, E3A, 4.88±0.49 mg/ml, E3B, 1.26±0.05 mg/ml, and E3C was 2.53±0.11 mg/ml. These results were compared to the standard Diclofenac diethylammonium, which had an IC_{50} value of 0.07±0.01 mg/ml. The results indicate that the most effective anti-inflammatory extract in the *in vitro* test was E1B with an IC_{50} value of 1.17±0.05 mg/ml. However, when compared to the standard Diclofenac diethylammonium, the anti-inflammatory effect of E1B was found to be significantly lower (p<0.05) as shown in **Table 2.**

Table 1 Physical characteristics of extracts from six spice samples

Samples	% yield	Characteristics of 1-hour extraction	Samples	% yield	Characteristics of 3-hours extraction
E1A	95.50%		E3A	90.00%	
E1B	96.00%		E3B	91.50%	
E1C	97.00%		E4C	91.00%	

Table 2 *In vitro* anti-inflammatory activity test results of the extracts

Samples	IC_{50} (mg/ml)
E1A	1.96±0.11*
E1B	1.17±0.05*
E1C	2.42±0.06*
E3A	4.88±0.49*
E3B	1.26±0.05*
E3C	2.53±0.11*
Diclofenac diethylammonium	0.07±0.01*

*A statistically significant difference (p<0.05) was observed when comparing the extraction time of the six extracts with Diclofenac diethylammonium.

The antibacterial activity against *S. mutans* was evaluated using the disc diffusion method. All six extract samples were tested and compared with the standard antibacterial agent Erythromycin. The results indicated that none of the six extracts inhibited the growth of *S. mutans* compared to the standard antibacterial agent erythromycin (0.015 mg), which produced a 23.23±0.17 mm inhibition zone. Therefore, it can be concluded that the six extract samples were ineffective in inhibiting *S. mutans* using the disc diffusion method, as shown in **Fig. 2-3.**

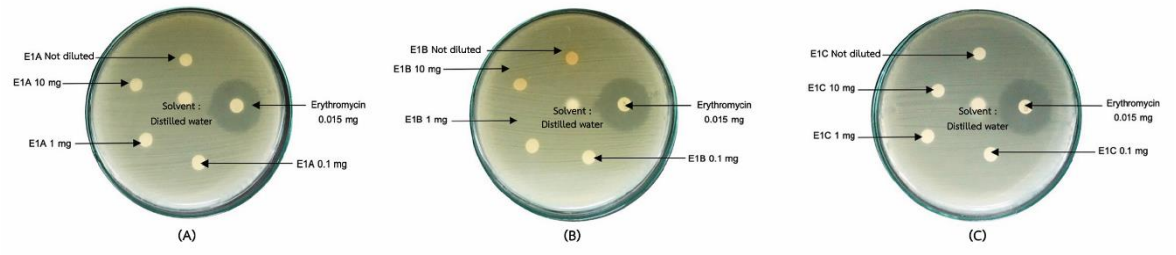

Figure 2 Comparison of the antibacterial activity of (A) E1A, (B) E1B, and (C) E1C against *S. mutans* using the disc diffusion method, with erythromycin as the standard drug

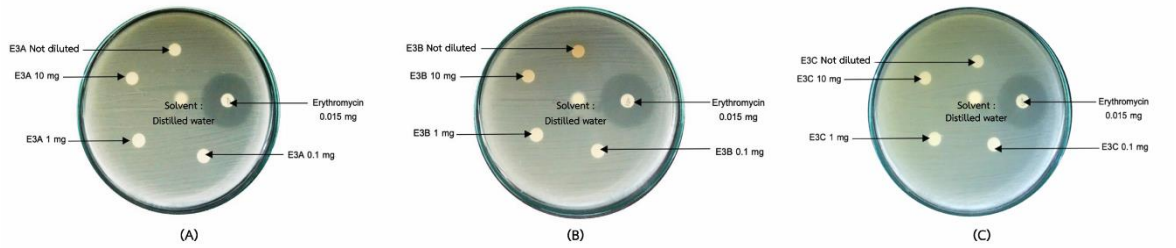

Figure 3 Comparison of the antibacterial activity of (A) E3A, (B) E3B, and (C) E3C against *S. mutans* using the disc diffusion method, with erythromycin as the standard drug

The total tannin contents of the six extract samples were analyzed using the Folin-Ciocalteu colorimetric method, with Tannic acid as the standard (see Table 3). From the results, it can be concluded that the extract E1B contains the highest total tannin content at 87.89±2.51 mg TAE/g extract, followed by E3B with 76.18±2.37 mg TAE/g extract. These two extracts are derived from the clove, which aligns with the results from the *in vitro* anti-inflammatory activity test, where E1B was found to have the most potent anti-inflammatory activity.

Table 3 Total tannin content analysis results.

Samples	Total tannin content (mg TAE/g extract)
E1A	7.78±0.10 [a*]
E1B	87.89±2.51 [b*]
E1C	8.62±0.20 [c*]
E3A	8.17±0.21 [a*]
E3B	76.18±2.37 [b*]
E3C	6.39±0.09 [c*]

*Letters a, b, and c show statistically significant differences between variables.

Discussion

The development of Thai spice extracts was based on the principles of the Thai traditional medicine water extraction method, specifically the decoction method. This study developed a laboratory extraction method using reflux extraction with water as the solvent. The water used in this extraction was potable water, which is safe for developing extracts intended for products that will be used in the human oral cavity. The 1 hr and 3 hr extraction times were based on traditional decoction methods (Phanpheng et al., 2021). The preliminary tests showed that shorter durations (e.g., 30–60 min) are effective for softer herbs. The 3-hour duration specifically allowed us to investigate if extended extraction improves yield or leads to degradation. Extracts showed different color intensities, but no scent difference, between 1 hr and 3 hr extraction times; the 3-hour extracts were consistently darker. Extended exposure to heat during the extraction process can lead to the degradation of sensitive active compounds, a phenomenon well-documented in scientific literature (Rizzo et al., 2020). This is why traditional extraction methods, such as decoction, frequently advise shorter extraction times, specifically to prevent the breakdown of thermolabile compounds, which are sensitive to heat, like tannins and flavonoids. By limiting heat exposure, the integrity of these beneficial compounds is preserved, thereby maintaining the overall efficacy of the extract. Among the six extract samples, E1B exhibited the highest *in vitro* anti-inflammatory activity, with an IC_{50} value of 1.17±0.05 mg/ml, which was significantly higher than that of the other samples (p<0.05). Additionally, E1A and E1C exhibited better anti-inflammatory effects than the extracts obtained with a 3-hour extraction time. Specifically, E1A had an IC_{50} value of 1.96±0.11 mg/ml and E3A had an IC_{50} value of 4.88±0.49 mg/ml, with statistically significant differences (p<0.05). Therefore, the extraction time of spices with water using this method is a critical factor that affects the quality of the extract in terms of its anti-inflammatory effect. If the extraction time exceeds 1 hr, it will significantly reduce the anti-inflammatory activity of the spice extract.

In this study, there was a limitation in that while the Folin-Ciocalteu method is generally used for total phenolic content, it served as a proxy for total tannin content because more specific quantification techniques (e.g., vanillin-HCl assay) were unavailable. The experimental results also revealed that E1B exhibited the highest total tannin content (87.89±2.51 mg TAE/g extract), correlating with its superior *in vitro* anti-inflammatory activity. Tannins, known for inhibiting inflammatory cytokines and enzymes such as COX-2 and LOX, are key bioactive compounds responsible for these effects. Conversely, the lower tannin levels in E1A and E1C likely explain their reduced anti-inflammatory activities. Tannin's ability to bind various organic molecules, including proteins and free radicals, further contributes to their anti-inflammatory and antibacterial properties (Chaiyasut & Nithitanakul, 2020; Preechatiwong & Ratanachamnong, 2021). While the extract's IC_{50} was higher than the standard, suggesting a comparatively lower potency, this presents a clear opportunity for optimization. Future research can focus on refining extraction methods or concentrating specific compounds to enhance their anti-inflammatory efficacy.

In this study, none of the six Thai spice water extracts showed antibacterial activity against *Streptococcus mutans* when tested using the disc diffusion method, in contrast to the standard antibiotic erythromycin, which produced a 23.23±0.17 mm inhibition zone. One possible explanation is that *S. mutans* possesses a dense and resilient cell wall structure, making it inherently resistant to certain natural extracts (Wongkum, 2019; Prachyakij & Punyaprateep, 2021; Preechatiwong & Ratanachamnong, 2021). Moreover, there has been limited research

on the antibacterial effects of water extracts from Thai spices against *S. mutans*. However, a study by Rahim and Khan (2006) found that both crude aqueous and solvent-based clove extracts were effective in reducing the cariogenic activities of *S. mutans*, such as their ability to adhere to surfaces, produce glucan, generate acid, and grow. Their findings revealed that while the solvent extract had stronger effects, the aqueous extract still demonstrated notable anti-cariogenic activity. This suggests that although the Thai spice water extracts in the present study did not exhibit direct antibacterial effects in the disc diffusion assay, they may still possess the potential to interfere with the virulence factors of *S. mutans* through other mechanisms. Further investigations using different methods or higher extract concentrations may be necessary to fully explore their therapeutic potential.

Conclusion and Suggestions

The purpose of this study was to test the Thai herbal extraction process, which involves boiling herbs in drinking water, to treat diseases. The test results showed that the extracts from Chinese cinnamon, clove, and star anise obtained through water extraction exhibited anti-inflammatory activity and contained a good amount of total tannins. Additionally, it can be concluded that the water extraction method of Thai spices, based on Thai traditional medicine knowledge, demonstrates that the extracts from the three spices have anti-inflammatory activity and a good total tannin content but cannot inhibit *S. mutans*. Extraction time was shown to affect both the anti-inflammatory activity and total tannin content. When comparing extraction times of 1 hr and 3 hr, the results showed that prolonged extraction time and accumulated temperature led to a decrease in the anti-inflammatory activity and total tannin content of the Thai spice extracts. For future research, the use of other safe solvents should be considered for developing human-use products to enhance the efficacy of Thai spice extracts. Additionally, the effects of combining these extracts with other herbal extracts should be studied to improve their therapeutic efficacy.

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Author Contributions

Author 1: Conceptualization, development and design of methodology, investigation, collection of data, data analysis and interpretation, and manuscript writing.
Author 2: Collection of data, data analysis and interpretation, and manuscript review and editing.
Author 3: Collection of data, data analysis and interpretation, and manuscript review and editing.
Author 4: Data analysis and interpretation, and manuscript review and editing.
Author 5: Data analysis and interpretation, and manuscript review and editing.

Conflict of Interests

All authors declare that they have no conflicts of interest.

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