Original Article

Unveiling the Botanical Riches: Enhancing Quality Control and Stability Assessment of Sa-Tri-Lhung-Klod Remedy Extract Through HPLC Profiling and Anti-inflammatory Potency Evaluation

Janjira Inprasit[1], Srisopa Ruangnoo[2*], Arunporn Itharat[2,3], Sunita Makchuchit[3], Weerachai Pipatrattanaseree[4], Neal M. Davies[5]

Abstract

Introduction: Sa-Tri-Lhung-Klod (ST) remedy is a Thai traditional medicine preparation, used for post-partum care including excreting amniotic fluid, reducing inflammation, and improving blood circulation. The standard quality values and the stability of ST have not been reported. Quality control and stability testing are necessary to determine and insure the standard quality of ST remedy.

Objectives: To evaluate the quality control of herbal components according to Thai Herbal Pharmacopoeia (THP). The stability of ST extracts was examined using high performance liquid chromatography (HPLC) and anti-inflammatory activity was measured.

Methods: The quality assessment of the ST and plant components was performed by moisture content, total ash, acid-insoluble ash, extractive value, and tannin content, following the THP method. The stability testing of ST extracts stored under accelerated conditions for six months was performed, and the percentage of the remaining compounds were evaluated using HPLC and tested for anti-inflammatory activity on nitric oxide (NO) inhibitory assay in RAW264.7 cells.

Results: Quality control of ST and plant components met THP requirements, and the tannin content of ST increased upon extraction. ST ethanolic extract (STE) and the remaining compounds exhibited stability after 6 months and anti-inflammatory activity on nitric oxide inhibition assay was retained from day 0 to day 180. However, ST aqueous extract (STW) was unstable from day 30.

Conclusions: The first report of quality control and stability testing of ST is outlined. ST and plant components comply with THP standard requirements. STE can be stored at room temperature for up to two years, whereas STW requires refrigeration in order to extend its shelf life.

Keywords: Sa-Tri-Lhung-Klod remedy, Quality control, Stability testing, HPLC, Anti-inflammatory activity

Volume 24, Issue 2, Page 45-58

https://asianmedjam.com

Received: 14 June 2023 **Revised: 9 Febuary 2024** **Accepted: 9 Febuary 2024**

[1] Student of Doctor of Philosophy Program in Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[2] Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[3] Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University, Pathum Thani, Thailand
[4] Regional Medical Science Center 12 Songkhla, Department of Medical Sciences, Songkhla, Thailand
[5] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada
***Corresponding author:** Srisopa Ruangnoo, Ph.D., Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand, Email: srisopa888@gmail.com, Tel. +66 2926 9734

Introduction

The Sa-Tri-Lhung-Klod (ST) remedy is a Thai traditional medicine remedy, which is described in the list of The National List of Essential Herbal Medicines (NLEM). It consists of seventeen herbs, including *Angelica sinensis* (Oliv.) Diels, *Artocarpus heterophyllus* Lam, *Caesalpinia sappan* Linn, *Carthamus tinctorius* Linn, *Curcuma comosa* Roxb, *Jasminum sambac* Ait, *Maclura cochinchinensis* (Lour.) Corner, *Mammea siamensis* Kosterm, *Mesua ferrea* Linn, *Mimusops elengi* L, *Nelumbo nucifera* Gaertn, *Piper longum* Linn, *Piper nigrum* Linn, *Piper ribesioides* Wall, *Piper sarmentosum* Roxb, *Plumbago indica* L, and *Salacia chinensis* L. The main medicinal taste of the ST is spicy, complemented with an astringent, sweet, and fragrant taste. Currently, ST remedy is prepared by decoction as an aqueous extract and maceration as an ethanolic extract in order to use for post-partum care and promote maternal health. Previously, clinical trials reported that ST is effective for uterus contraction, amniotic fluid excretion, and maintenance of overall health after childbirth.[1] In addition, ST extract has been reported to demonstrate biological activities, e.g., anti-inflammatory activity on cyclooxygenase-2 enzyme (Cox-2) inhibition and cytotoxic activity on cervical and ovarian cancer cells.[2,3]

However, standardization and quality control of the ST remedy and its constituent plant components are important to assess and determine their quality and stability for use. Quality control and stability testing are performed to guide the development of herbal pharmacopeia, which includes the correct selection of herbs, the development of quality standards, and determination of the shelf-life of herbal extracts, which varies with time under the influence of a variety of environmental factors such as temperature, light, and humidity.[4] Stability studies ensure the quality of active ingredients and predict the shelf life of herbal formulations. Storage conditions can also be recommended.[5] To date, there have been no monograph reports on quality control and stability studies of the ST remedy in Thai Herbal Preparation Pharmacopoeia (THPP).[6] However, the standardized monographs in the THP appear for 11 plant components listed in ST remedy, such as *Angelica sinensis*, *Carthamus tinctorius*, *Curcuma comosa*, *Jasminum sambac*, *Mesua ferrea*, *Mimusops elengi*, *Nelumbo nucifera*, *Piper longum*, *Piper nigrum*, *Piper ribesioides*, and *Piper sarmentosum*.[7] The quality assessment of ST remedy is important to ensure further acceptance in the modern medical system. Therefore, it is necessary to evaluate the quality control and stability analysis of the ST remedy accordingly. This study aims to determine the quality control of the ST remedy and plant components, including moisture content, total ash, acid-insoluble ash, extractive value, and tannin content, following the Thai Herbal Pharmacopoeia (THP) method. Additionally, the stability of the ST extracts under accelerated storage conditions was evaluated by determining the percentage of remaining compounds using HPLC and tested for anti-inflammatory activity on nitric oxide (NO) inhibitory assay in RAW264.7 cells.

Methods

Chemicals and Reagents

Dimethyl sulfoxide (DMSO) and isopropanol were purchased from RCl Labscan, Thailand. Fetal bovine serum (FBS), penicillin-streptomycin (P/S), RPMI medium 1640 (BIOCHROM^{AG}), Trypan blue stain 0.4%, and Trypsin-EDTA were purchased from Gibco, USA. Lipopolysaccharide from *E. coli* 055:B5 (LPS), *N*-(1-Naphthyl) ethylenediamine dihydrochloride, phosphoric acid (H_3PO_4), sulfanilamide, and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, USA. Phosphate buffered saline (PBS) was purchased from Biochrom, Germany. Hide powder was purchased from Eurofins BLC Leather Technology Centre Limited, UK.

Plant Material

Sa-Tri-Lhung-Klod remedy (ST) consists of 17 different herbs. **Table 1** shows the botanical name, family, Thai name, collected from, voucher specimens, part used, and proportion used. The voucher specimens were obtained at the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand.

Extraction

Each plant was washed in water, sliced into small pieces, dried in an oven at a temperature of 50 °C, and pulverized into a coarse powder. Then they were weighed and mixed to make the ST according to NLEM. The proportion of maceration and decoction was 1 g of the coarse powder with 20 mL of 95% ethanol and distilled water, respectively. ST was macerated with 95% ethanol for 3 days/3 times and concentrated to dryness by an evaporator (Rotavapor R-205, Germany), obtaining an 8.51% weight per weight (w/w) yield of ethanolic extract of ST (STE). For the aqueous extract, ST was boiled for 30 min in distilled water at the boiling point and concentrated to dryness by lyophilizer (Lyophilization System Inc, USA). An aqueous extract of ST (STW) was obtained at 12.34% w/w yield.

Assays for Quality Control

Loss on Drying[7]

Two grams of sample powder were put into an electronic moisture analyzer (Scaltec instrument, Germany) at 105 °C until a constant weight was obtained. Each sample was examined in triplicate and the mean weight loss on drying was reported.

$$\% \text{ Loss on drying} = \frac{\textit{Start weight (g)-End weight (g)}}{\textit{Start weight (g)}} \times 100$$

Total Ash[7]

A crucible was prepared by putting it in a hot air oven at 105 °C until the weight was stable. Then, two grams of sample powder are put in the crucible and burned using a muffle furnace (Nabertherm, Germany) at 450 °C for 9 hours. Next, the crucible was cooled in a desiccator and then weighed. This process was repeated except it was heated for 5 hours instead of nine until the weight of the crucible and ash was constant.

$$\% \text{ Total ash} = \frac{\textit{Stable weight after burning (g)}}{\textit{Weight before burning (g)}} \times 100$$

Acid-insoluble Ash[7]

After the weight of total ash was determined to be constant the total ash was boiled in 25 mL of 10% HCl for 5 minutes. After cooling, it was filtered through Whatman paper no.42. The ash in the paper was washed with hot water until the filtrate was neutral, then burned in a muffle furnace at 500 °C for 9 hours. This process is repeated until the crucible's weight is stable.

$$\% \text{ Acid-insoluble ash} = \frac{\textit{Stable weight after burning (g)}}{\textit{Weight before burning (g)}} \times 100$$

Ethanol-soluble Extractive Value[7]

Sample powder (5 grams) was macerated in 100 mL of 95% ethanol for 24 hours, shaking frequently during the first 6 hours, and then allowed to stand for 18 hours. The plant extract was filtered and 20 mL filtrate was put in a shallow dish. Then, the extract was dried at 105 °C until constant weight.

$$\% \text{ Ethanol-soluble extractive} = \frac{\textit{Weight of the extract (g)}}{\textit{Weight of dried powder (g)}} \times 100$$

Water-soluble Extractive Value[7]

The procedure is similar to the ethanol-soluble extractive value method but uses 0.25% chloroform in water instead of ethanol.

$$\% \text{ Water-soluble extractive} = \frac{\textit{Weight of the extract (g)}}{\textit{Weight of dried powder (g)}} \times 100$$

Determination of Tannins[7]

Tannins testing used the gravimetric technique. Coarse powder (STP), aqueous extract (STW), and 95% ethanolic extract (STE) of ST were the substances employed to determine tannins. Four grams of each sample were accurately weighed, 150 mL of distilled water was added, and the samples were heated in a water bath at 80 °C for 30 minutes. The mixture was transferred quantitatively to a 250 mL volumetric flask and diluted to volume with distilled water.

Determination of total water-extractives: 50 mL of the sample was evaporated. The residue was weighed after 4 hours of drying at 105 °C (*T1*).

Determination of water-extractives not bound with hide powder: hide powder (6 g) was added to 80 mL of sample and shaken for 1 hour. After filtering and evaporating 50 mL of the filtrate was completely dried. The residue was weighed after 4 hours of drying at 105 °C (*T2*).

Determination of water-soluble hind powder: hide powder (6 g) was added to 80 mL of distilled water and shaken for 1 hour. Subsequently, the same process was followed with T_2 (*T0*).

Calculate the percentage of tannins from the expression:

$$\% \text{ Tannins} = \frac{[(T1-T2+T0)\ x\ 5]}{W} \times 100$$

Where, W is the weight in grams of the substance taken, calculated on a dried basis.

Stability Testing [8]

The purpose of drug stability testing is to control the quality and storage methods to maintain therapeutic efficacy. STE and STW were exposed for six months, under 40 ± 2 ºC with 75 ± 5% relative humidity (RH) as the accelerated condition. The samples were sampled on days 0, 15, 30, 60, 90, 120, 150, and 180. All samples were determined for the percentage of remaining compounds using HPLC and tested for anti-inflammatory activity on NO inhibitory effect in RAW264.7 cells.

Determination of the Remaining Compounds of ST Remedy Extracts Using HPLC

Brazilin, (3*S*)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol, Piperine, Pipernonaline, (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol, (3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadiene-3-ol, and Artocarpin are the main chemical compounds determined in ST remedy. These compounds showed peaks which can be integrated at RT 5.433, RT 23.519, RT 25.136, RT 31.585, RT 32.160, RT 33.063, and RT 35.687, respectively.[9] The stability analysis of these compounds was conducted using an Agilent 1200 HPLC system (Agilent Technologies, USA), with a diode array detector and automatic injector. A Phenomenex Luna 5u C18(2) 100A column (250 × 4.6 mm) (Phenomenex, USA) was used for chromatographic separation. Agilent ChemStation software was used to analyze the data. The mobile phase was composed of acetonitrile: water (v/v) with the following gradient elution: 0 min-20 : 80 v/v, 30 min-5 : 95 v/v, and 40 min 10 seconds-20 : 80 v/v. The flow rate was set at 1.0 mL/min with an injection volume of 10 µg/mL and the total run time of analysis was 45 min. A diode array detector was set at 210 nm.

STE and STW were prepared by dissolving them with methanol and deionized water at a concentration of 5 mg/mL, respectively. The solution was filtered through 0.45 µM nylon membrane filters. 10 µL of sample solutions were injected into the HPLC column and separated under the above chromatographic condition. The mean peak areas (mAU) of each chemical compound were calculated as the percentage of remaining compounds, with Day 0 set to 100%.

Anti-inflammatory Activity on Nitric Oxide (NO) Inhibitory Assay[10]

STE was dissolved in sterile DMSO to a concentration of 50 mg/mL. STW was dissolved to a concentration of 10 mg/mL in sterile deionized water and filtered through a Millipore 0.22 µM filter. STE and STW were diluted in the cultured medium to obtain a final concentration of 1-100 µg/mL.

RAW 264.7 cells (ATCC® TIB-71™) were obtained from the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Thammasat University, Pathum Thani, Thailand. The cells were grown in RPMI 1640 medium containing 10% FBS, and 1% P/S in an incubator with 5% CO_2 at 37 °C. Cells were seeded in 96-well plates at $1x10^5$ cells/well and incubated for 24 hours. Then, cells were stimulated with lipopolysaccharide (LPS) (10 ng/mL)and treated with extracts at various concentrations for 24 hours. Later, the supernatant was collected to detect NO production using the Griess reagent [1% sulfanilamide/0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride, each in 2.5% H_3PO_4]. The optical density (OD) was measured using a microplate reader (Bio Tex, USA) at 570 nm.

MTT assay was used to measure cell viability. MTT solution (10 µL, 5 mg/mL in PBS) was added to the wells without LPS and incubated for 2 hours. Subsequently, isopropanol containing 0.04 M HCl was added to dissolve the formazan production. The OD was measured at 570 nm. No cytotoxicity was observed when the survival rate was greater than 70% compared with the control.

Statistical Analysis

The data were performed in triplicate and the results were presented as mean ± standard Deviation (SD). Statistical analysis was determined by one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests using the SPSS

program (IBM SPSS Statistics 26). Statistical significance was indicated when the *p*-value < 0.05.

Results

Quality Control

Physical standardization of ST remedy and plant components were investigated as follows: loss on drying (moisture content), extractive value, total ash, and acid-insoluble ash for inorganic contamination. The quality of the plants complied with standard values of Thai Herbal Pharmacopoeia loss on drying < 10%, total ash < 10%, and acid-insoluble ash < 2%. The results of the quality control of the ST and its ingredients are shown in **Table 2**. The percentage of loss on drying of ST remedy was 9.17 ± 0.04% w/w. *Angelica sinensis* showed the highest percentage of loss on drying with 9.84 ± 0.14% w/w, while *Piper longum* had the lowest percentage with 6.45 ± 0.68% w/w.

The percentage of total ash and acid-insoluble ash of ST remedy were 6.37 ± 0.18 and 1.03 ± 0.09% w/w, respectively. Among all the samples tested, *Mammea siamensis* showed the highest percentage of total ash (8.16 ± 0.14% w/w) and *Plumbago indica* exhibited the highest percentage of acid-insoluble ash (1.75 ± 0.03% w/w). On the other hand, *Caesalpinia sappan* demonstrated the lowest in total and acid-insoluble ash (1.03 ± 0.06 and 0.07 ± 0.01% w/w).

The percentage of ethanol- and water-soluble extractive values of ST remedy were 1.69 ± 0.09 and 2.99 ± 0.40%, respectively. *Mesua ferrea* had the highest ethanol-soluble extractive value of 3.35 ± 0.28%, while *Angelica sinensis* had the highest percentage of water-soluble extractive value of 9.87 ± 0.01%. However, *Artocarpus heterophyllus* showed the lowest percentage in both ethanol- and water-soluble extractive values (0.21 ± 0.04 and 0.45 ± 0.03%, respectively).

The results revealed that the quality control of ST and plant components met THP requirements. The result of tannin content in the ST remedy is presented in **Figure 1**. A coarse powder of ST (STP) had the lowest tannin content of 2.48 ± 2.14%, whereas the tannin content obtained in ST extracts (STW and STE) gave higher values than that of STP. The highest values of tannins are found in STW. It provided a tannin content of 5.38 ± 2.58%. STE had a tannin content of 4.14 ± 0.71%. These results showed that the tannin content was increased upon extraction, especially the aqueous extraction.

Stability Testing of STE and STW Storage under Accelerated Conditions

The stability testing results of STE and STW storage under accelerated conditions at day 0, 15, 30, 60, 90, 120, 150, and 180 on the percentage of remaining compounds and inhibitory effects of LPS-induced NO production in RAW-264.7 cells are shown in **Table 3**.

Percentage of Remaining Compounds of STE and STW

STE and STW used the same chromatographic conditions to determine the chemical characteristics of their crude extracts as HPLC fingerprints (see **Figure 2**). STE demonstrated 7 main chemical compounds. Brazilin was obtained from *Caesalpinia sappan*. Piperine was obtained from *Piper nigrum* and *Piper longum*. In addition, *Piper longum* also provided Pipernonaline. Subsequently, (3*S*)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol, (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol, and (3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadiene-3-ol were derived from *Curcuma comosa*. Finally, Artocarpin was obtained from *Artocarpus heterophyllus*. For STW, the chemical compound was determined to be Brazilin.

The stability under accelerated conditions of ST remedy extracts was examined in the percentage of the various remaining compounds using HPLC. The results are shown in **Figure 2** and **Table 3**. The results showed that the seven main compounds of STE were stable from day 0 to day 180. Nevertheless, Brazilin, a compound of STW was not stable and was not detectable from day 30. Therefore, HPLC fingerprints of ST extracts can be used to verify drug stability and quality control.

Anti-inflammatory Activity on NO

Inhibition

The stability of ST extracts was tested with the anti-inflammatory activity against LPS-induced NO production and survival of RAW264.7 cells. As the results are shown in **Figure 3** and **Table 3**. Day 0 of STE showed anti-inflammatory values with IC_{50} of 20.59 ± 0.03 μg/mL and also showed similar values all under accelerated conditions from day 0 to day 180 (IC_{50} range of 20.59 to 25.61 μg/mL). However, from day 15 to day 180 STE showed a statistically significant difference compared to day 0 ($p < 0.05$). STW, Day 0 displayed an IC_{50} of 52.93 ± 0.90 μg/mL, and Day 15 to Day 180 showed an IC_{50} of more than 100 μg/mL. In addition, the cytotoxicity of all extracts was examined using the MTT assay to ensure that the inhibitory effect of NO production was not the result of cell death. The results showed that none of the extracts were toxic to RAW264.7 cells.

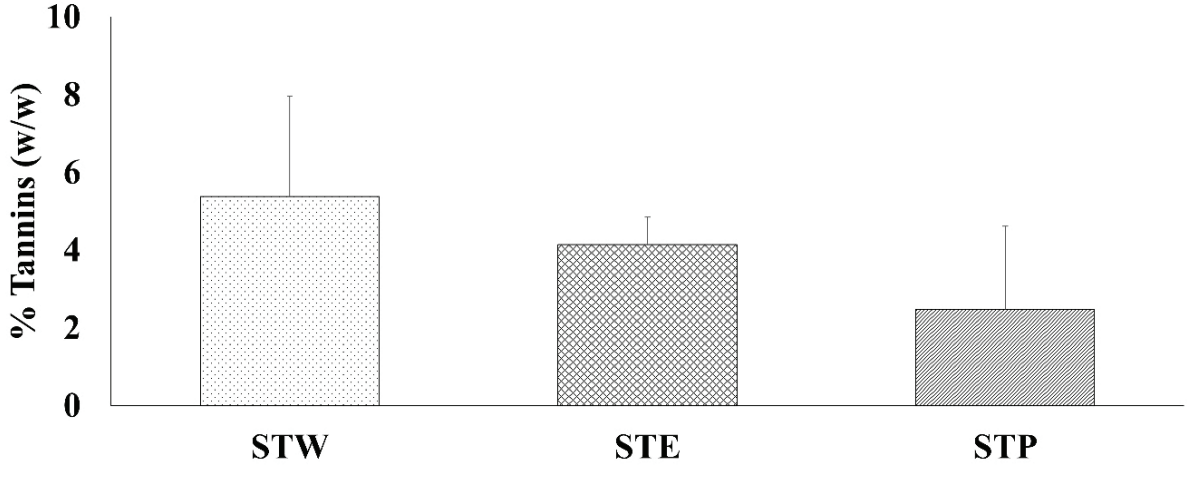
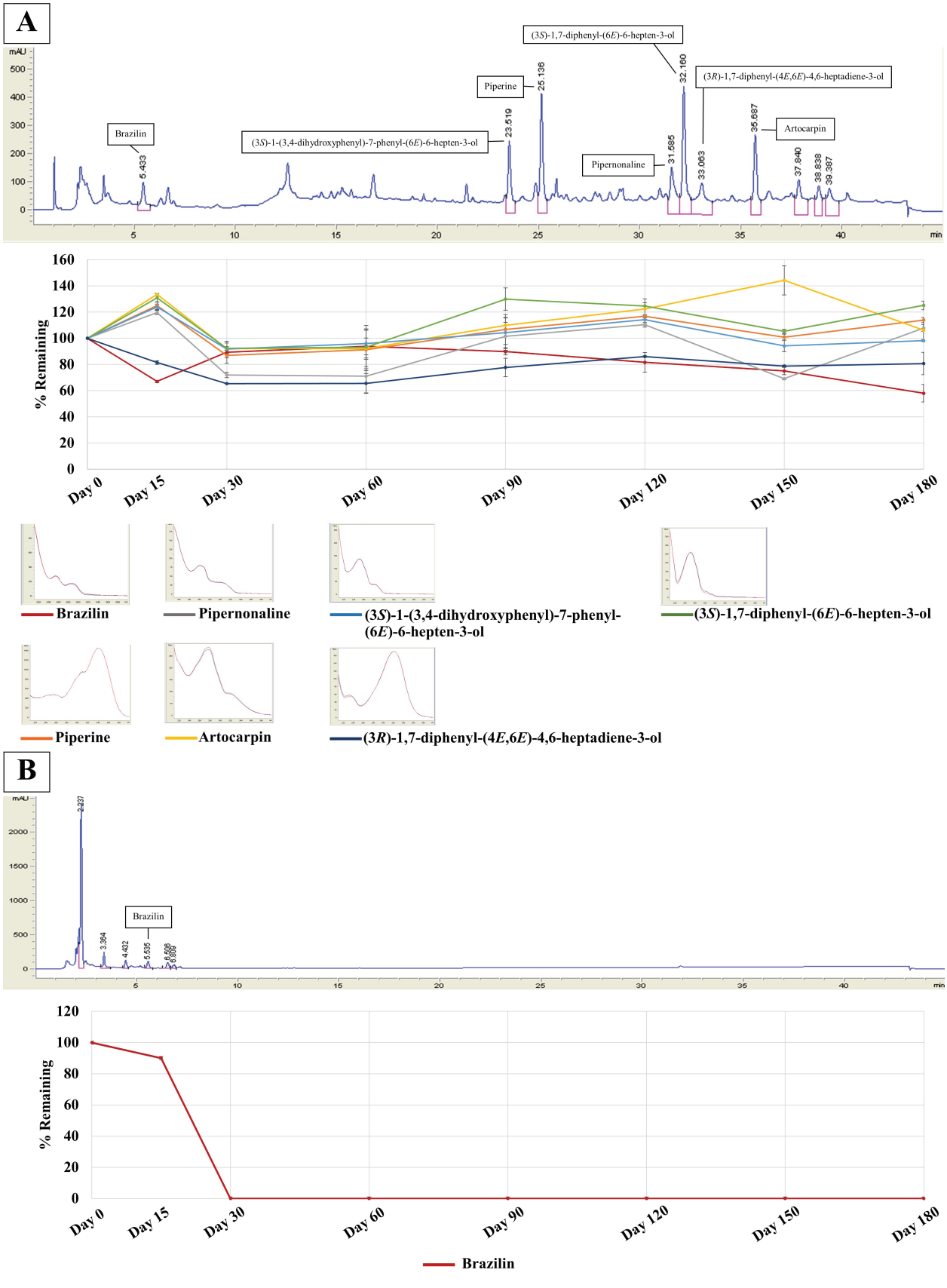


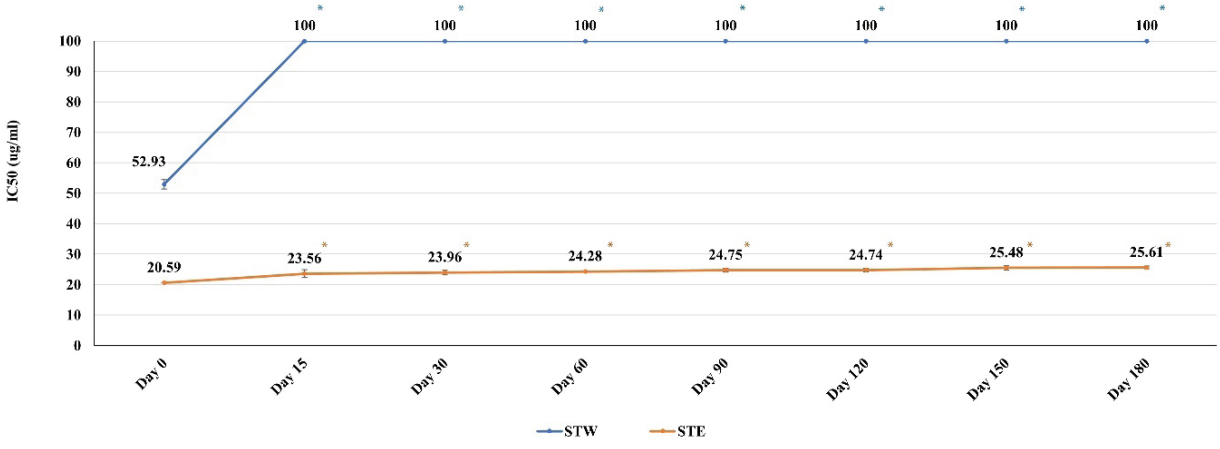
Figure 1 The percentage of tannins obtained from the Sa-Tri-Lhung-Klod remedy. Each value represents the mean ± SD., n = 3 for each group. STW: aqueous extract of Sa-Tri-Lhung-Klod remedy, STE: 95% ethanolic extract of Sa-Tri-Lhung-Klod remedy, and STP: coarse powder of Sa-Tri-Lhung-Klod remedy.

Figure 2 HPLC chromatogram and the percentage of the various remaining compounds of (A) STE and (B)



STW (n = 3).

Figure 3 The stability of anti-inflammatory activity (IC_{50}) of the STE and STW under accelerated condition



at 40 ± 2 ºC, 75 ± 5% RH for 6 months at different times (n = 3). * $p < 0.05$ compared with Day 0 in the same sample. The positive control was indomethacin (IC_{50} = 28.37 ± 0.28 µg/mL).

Table 1 Component plants and their parts in Sa-Tri-Lhung-Klod remedy

Botanical name	Family	Thai name	Collected from	Voucher specimen number	Part used	Proportion (% w/w)
Angelica sinensis (Oliv.) Diels	UMBELLIFERAE	Kot-Chiang	China	SKP199010901	Root	7.4
Artocarpus heterophyllus Lam.	MORACEAE	Kha-Nun	Nakhon Ratchasima	SKP117010801	Stem	7.4
Caesalpinia sappan Linn.	LEGUMINOSAE	Fang-Sen	Bangkok	SKP098031901	Stem	7.4
Carthamus tinctorius Linn.	COMPOSITAE	Kham-Foi	Chiang Mai	SKP051032001	Flower	3.7
Curcuma comosa Roxb.	ZINGIBERACEAE	Wan-Chak-Mod-Look	Phetchabun	SKP201030301	Rhizome	7.4
Jasminum sambac Ait.	OLEACEAE	Ma-Li	Nakhon Pathom	SKP129101901	Flower	3.7
Maclura cochinchinensis (Lour.) Corner	MORACEAE	Kae-Lae	Prachuap Khiri Khan	SKP117130301	Stem	7.4
Mammea siamensis Kosterm.	GUTTIFERAE	Sa-Ra-Pee	Ratchaburi	SKP083131901	Flower	3.7
Mesua ferrea Linn.	GUTTIFERAE	Boon-Nak	Ratchaburi	SKP083130601	Flower	3.7
Mimusops elengi L.	SAPOTACEAE	Phi-Kul	Ratchaburi	SKP171130501	Flower	3.7
Nelumbo nucifera Gaertn.	NELUMBONACEAE	Bua-Luang	Ratchaburi	SKP125141401	Pollen	3.7
Piper longum Linn.	PIPERACEAE	Di-Plee	Chanthaburi	SKP146160301	Fruit	7.4
Piper nigrum Linn.	PIPERACEAE	Prik-Thai	Chanthaburi	SKP146161401	Fruit	7.4
Piper ribesioides Wall.	PIPERACEAE	Sa-Kan	Sakon Nakhon	SKP146161801	Stem	7.4
Piper sarmentosum Roxb.	PIPERACEAE	Cha-Phlu	Ratchaburi	SKP146161901	Root	3.7
Plumbago indica L.	PLUMBAGINACEAE	Chet-Ta-Mun-Phloeng-Daeng	Bangkok	SKP148160901	Root	7.4
Salacia chinensis L.	CELASTRACEAE	Kam-Phaeng-Chet-Chan	Ratchaburi	SKP044190301	Stem	7.4

Table 2 Quality control of Sa-Tri-Lhung-Klod remedy and its plant ingredients (n = 3)

Botanical name	% Loss on drying (w/w)		% Ash content (w/w)				% Extractive value (w/w)	
			Total ash		Acid-insoluble ash		95% EtOH soluble	Water soluble
	Mean ± SD	Limit	Mean ± SD	Limit	Mean ± SD	Limit	Mean ± SD	Mean ± SD
Angelica sinensis	9.84 ± 0.14	≤ 10	7.07 ± 0.28	≤ 7 [a]	1.71 ± 0.04	≤ 2 [a]	2.55 ± 0.03	9.87 ± 0.01
Artocarpus heterophyllus	8.51 ± 0.86	≤ 10	1.64 ± 0.09	≤ 10	0.63 ± 0.04	≤ 2	0.21 ± 0.04	0.45 ± 0.03
Caesalpinia sappan	8.34 ± 0.15	≤ 10	1.03 ± 0.10	≤ 10	0.07 ± 0.01	≤ 2	0.26 ± 0.02	0.47 ± 0.09
Carthamus tinctorius	9.68 ± 0.24	≤ 14 [a]	7.68 ± 0.22	≤ 15 [a]	1.69 ± 0.02	≤ 5 [a]	2.76 ± 0.08	5.90 ± 0.10
Curcuma comosa	9.66 ± 0.21	≤ 11 [a]	5.02 ± 0.47	≤ 10 [a]	1.50 ± 0.12	≤ 3 [a]	1.33 ± 0.12	2.63 ± 0.10
Jasminum sambac	9.74 ± 0.20	≤ 11 [a]	8.00 ± 0.46	≤ 8 [a]	0.36 ± 0.06	≤ 1.5 [a]	1.42 ± 0.15	5.16 ± 0.26
Maclura cochinchinensis	9.55 ± 0.31	≤ 10	4.79 ± 0.10	≤ 10	0.21 ± 0.05	≤ 2	0.28 ± 0.03	0.63 ± 0.05
Mammea siamensis	8.41 ± 0.14	≤ 10	8.16 ± 0.24	≤ 10	0.56 ± 0.03	≤ 2	2.25 ± 0.07	3.73 ± 0.02
Mesua ferrea	9.41 ± 0.15	≤ 11 [a]	3.41 ± 0.36	≤ 5 [a]	0.59 ± 0.17	≤ 1.5 [a]	3.35 ± 0.28	3.03 ± 0.32
Mimusops elengi	9.48 ± 0.08	≤ 16 [a]	5.43 ± 0.24	≤ 7 [a]	1.15 ± 0.30	≤ 3 [a]	0.28 ± 0.02	1.88 ± 0.14
Nelumbo nucifera	8.04 ± 0.04	≤ 12 [a]	4.36 ± 0.05	≤ 6 [a]	0.30 ± 0.02	≤ 1 [a]	1.67 ± 0.06	2.92 ± 0.03
Piper longum	6.45 ± 0.68	≤ 10	5.48 ± 0.54	≤ 7.5 [a]	0.28 ± 0.04	≤ 0.4 [a]	1.76 ± 0.09	2.60 ± 0.11
Piper nigrum	8.20 ± 0.02	≤ 10	4.44 ± 0.19	≤ 4 [a]	0.39 ± 0.04	≤ 0.5 [a]	1.03 ± 0.01	0.52 ± 0.07
Piper ribesioides	7.85 ± 0.46	≤ 8 [a]	3.73 ± 0.13	≤ 9 [a]	0.27 ± 0.04	≤ 2	0.76 ± 0.04	1.54 ± 0.04
Piper sarmentosum	7.91 ± 1.09	≤ 10 [a]	6.82 ± 0.27	≤ 14 [a]	0.23 ± 0.01	≤ 4 [a]	0.46 ± 0.06	2.60 ± 0.14
Plumbago indica	9.39 ± 0.36	≤ 10	6.75 ± 0.28	≤ 10	1.75 ± 0.04	≤ 2	0.34 ± 0.06	5.44 ± 0.16
Salacia chinensis	9.25 ± 0.25	≤ 10	2.24 ± 0.03	≤ 10	0.15 ± 0.03	≤ 2	1.09 ± 0.08	0.89 ± 0.03
Sa-Tri-Lhung-Klod remedy	9.17 ± 0.04	-	6.37 ± 0.30	-	1.03 ± 0.15	-	1.69 ± 0.09	2.99 ± 0.40

[a] The standard values of THP 2021, - Not reported

Table 3 Stability of STE and STW on the percentage of the remaining compounds and inhibitory effects of LPS-induced NO production during 180-day storage under accelerated conditions. Each value represents the mean ± SD (n = 3).

Sample	Day 0	Day 15	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
% Remaining compounds (%)								
STE:								
Brazilin	100.00 ± 0.00	67.01 ± 0.87*	89.31 ± 8.46*	93.82 ± 6.44	89.81 ± 2.42*	81.65 ± 7.50*	75.03 ± 2.73*	57.97 ± 6.70*
(3*S*)-1-(3,4-dihydroxyphenyl) -7-phenyl-(6*E*)-6-hepten-3-ol	100.00 ± 0.00	123.95 ± 2.14	91.73 ± 0.96	95.98 ± 10.97	104.35 ± 11.52	144.21 ± 3.45	94.20 ± 4.42	98.19 ± 0.72
Piperine	100.00 ± 0.00	125.07 ± 2.86	86.86 ± 1.78	91.27 ± 15.93	106.79 ± 11.44	116.89 ± 1.12	100.88 ± 2.38	113.95 ± 1.98
Pipernonaline	100.00 ± 0.00	119.70 ± 1.60	71.89 ± 2.01*	71.09 ± 12.79*	101.57 ± 10.71	110.36 ± 1.76	69.10 ± 0.66*	107.84 ± 2.65
(3*S*)-1,7-diphenyl-(6E)-6-hepten-3-ol	100.00 ± 0.00	131.01 ± 3.28	92.28 ± 1.41	93.20 ± 16.52	129.90 ± 8.53	124.72 ± 2.48	105.43 ± 1.48	125.10 ± 3.37
(3*R*)-1,7-diphenyl-(4*E*,6*E*)-4,6-heptadiene-3-ol	100.00 ± 0.00	81.45 ± 1.43*	65.37 ± 0.19*	65.60 ± 7.56*	77.73 ± 7.04*	86.09 ± 0.57*	78.81 ± 0.53*	80.71 ± 8.55*
Artocarpin	100.00 ± 0.00	133.48 ± 0.63	92.06 ± 4.40	92.19 ± 13.88	109.92 ± 5.68	122.36 ± 7.82	144.27 ± 11.13	106.16 ± 7.15
STW:								
Brazilin	100.00 ± 0.00	90.08 ± 1.46*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*
IC_{50} of NO production (µg/mL)								
STE	20.59 ± 0.06	23.56 ± 1.30*	23.96 ± 0.79*	24.28 ± 0.20*	24.75 ± 0.64*	24.74 ± 0.60*	25.48 ± 0.74*	25.61 ± 0.45*
STW	52.93 ± 1.55	> 100 *	> 100 *	> 100 *	> 100 *	> 100 *	> 100 *	> 100 *
Indomethacin	28.37 ± 0.28	-	-	-	-	-	-	-

* $p < 0.05$ compared with Day 0 in the same sample. – Not test

Discussion

The use of herbal medicines in Thai traditional medicine plays an important role in maternal health care during the post-partum period. It is interesting that the ST remedy is currently included in the NLEM and is used in obstetrics and gynecology to reduce inflammation, excrete amniotic fluid, and nourish the blood. The Thai Herbal Preparation Pharmacopoeia (THPP) provides five monographs on obstetrics and gynecology medicine remedies, including Fai-Ha-Kong, Fai-Pra-Lai-Kan, Lueat-Ngam, Pluk-Fai-That, and Pra-Sa-Phlai remedy.[6] However, the THPP lacks a monograph for the quality control and stability study of the ST remedy. This study provides new scientific evidence on the quality control and stability of ST remedy, following THP guidelines.The findings of this research can support the establishment of a quality specification for the ST remedy.

Quality control of ST remedy and plant components was investigated according to THP as follows: loss on drying, total ash, acid-insoluble ash, and extractive values. The standard requirements on quality control are only available for some plants listed in ST remedy in THP such as *Angelica sinensis*, *Carthamus tinctorius*, *Curcuma comosa*, *Jasminum sambac*, *Mesua ferrea*, *Mimusops elengi*, *Nelumbo nucifera*, *Piper longum*, *Piper nigrum*, *Piper ribesioides*, and *Piper sarmentosum*.[7] Loss on drying is the weight loss due to water and any other volatile matter that can be driven off. The standard quality value of a loss on drying in the THP exhibited *Carthamus tinctorius*, *Curcuma comosa*, *Jasminum sambac*, *Mesua ferrea*, *Mimusops elengi*, *Nelumbo nucifera*, *Piper ribesioides*, and *Piper sarmentosum* was not more than 14.0, 11.0, 11.0, 11.0, 16.0, 12.0, 8.0, and 10.0% w/w, respectively.[7] Total ash is what remains after the sample has completely burned, which is obtained from the plant tissue itself and extraneous matter (soil and sand) adhering to the plant surface. The total ash standards in THP of *Angelica sinensis*, *Carthamus tinctorius*, *Curcuma comosa*, *Jasminum sambac*, *Mesua ferrea*, *Mimusops elengi*, *Nelumbo nucifera*, *Piper longum*, *Piper nigrum*, *Piper ribesioides*, and *Piper sarmentosum* are not exceed 7.0, 15.0, 10.0, 8.0, 5.0, 7.0, 6.0, 7.5, 4.0, 9.0, and 14.0% w/w respectively.[7] Acid-insoluble ash is the portion of ash that is insoluble in acid. It is a part of the total ash produced when a test material is burned. An ash constitutes inorganic matter, but acid-insoluble ash consists mainly of silica.[12] The standard values of acid-insoluble ash of some herbs in THP such as *Angelica sinensis*, *Carthamus tinctorius*, *Curcuma comosa*, *Jasminum sambac*, *Mesua ferrea*, *Mimusops elengi*, *Nelumbo nucifera*, *Piper longum*, *Piper nigrum*, and *Piper sarmentosum* were set not more than 2.0, 5.0, 3.0, 1.5, 1.5, 3.0, 1.0, 0.4, 0.5, and 4.0% w/w respectively.[7] However, there is no specific requirement for ST remedy in THPP, the general requirements of loss on drying, total ash, and acid-insoluble ash for the herbal plants should not exceed 10%, 10%, and 2%, respectively.[11] According to the results, the ST remedy and all plant components have met the standard criteria of loss on drying, total ash, and acid-insoluble ash.

Extractive values of a medicinal plant are used to determine amounts of active constituents present in a given amount of plant material when extracted. This is typically done using a solvent to extract the active compounds from the plant material. The extractive values can be an important factor in determining the potency and effectiveness of herbal remedies and medicines. Tannins are commonly found in plants and they are important bioactive compounds reported to have several biological effects, including anti-inflammatory activity. Tannins are used as anti-inflammatory agents and have wound-healing potential.[13,14] In addition, tannins contribute directly to major organoleptic properties, in particular to taste attributes such as astringency and bitter taste.[15] ST contains astringent taste plants derived from the heartwood, namely *Artocarpus heterophyllus*, *Caesalpinia sappan*, *Maclura cochinchinensis*, and *Salacia chinensis*. The present results suggest that both STE and STW resulted in higher tannin concentrations than the coarse powder. As a result, the extraction process can enhance the tannin concentration. Unfortunately, ST remedy and plant components have not reported tannin content in THPP and THP.

Stability testing was performed to control the quality and storage methods to maintain its effectiveness following the ICH guidelines.[8] Previous research has reported on the chemical compounds of STE and STW. In addition,

STE and STW exhibited anti-inflammatory effects by inhibiting the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2).[9] Additionally, among the compounds tested in previous research, Brazilin, Piperine, (3*S*)-1-(3, 4-dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol, (3*S*)-1,7-diphenyl-(6*E*)-6-hepten-3-ol, and (3*R*)-1, 7-diphenyl-(4*E*,6*E*)-4,6-heptadiene-3-ol were effective against lipopolysaccharide (LPS)-induced NO production in RAW264.7 cells.[16-18] Stability testing from this study indicated that the percentage of the remaining compounds of STE storage under accelerated conditions remained constant from day 0 to day 180 and did not lose its inhibitory effect on NO production. In contrast, the percentage of remaining brazilin of STW was unstable and undetectable from day 30 and anti-inflammatory effects were not measurable. After STW storage under accelerated conditions at 40 ± 2 ºC, 75 ± 5% RH from day 15-180, its physical characteristics were moist and sticky, which differed from the fresh extract (day 0) as a completely dry powder. Humidity and temperature may cause STW to deteriorate and cause the brazilin content to degrade. Therefore, it can be concluded that STE can be stored at room temperature for up to two years, whereas STW requires refrigeration in order to extend its shelf life.

This study highlights the importance of quality control and stability testing for ST remedy. The findings can serve as a benchmark for future scientific research and manufacturing, ensuring consistent quality and shelf-life of ST remedy. It is essential to adhere to these standards to maintain the efficacy of the remedy for patient use.

Financial Support

This work was supported by the Thailand Science Research and Innovation Fundamental Fund (TUFF28/2565) and the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University.

Compliance with Ethics Requirements

The experiments were approved by the Institute Biosafety Committee of Thammasat University (Number 065/2561) and performed under biosafety level 2.

Conflict of Interest

The authors declare that they have no conflict of interests.

Acknowledgments

The authors are grateful to the Thailand Science Research and Innovation Fundamental Fund, the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), and Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, for supporting and providing the laboratory facilities to complete this project. Dr. Davies acknowledges the Buuluan ASEAN Chair Professorship at Thammasat University.

Author Contributions

J. Inprasit undertook all the laboratory experiments and wrote the draft manuscript with support from **S. Ruangnoo** and **A. Itharat**, who were J. Inprasit's advisors. **A. Itharat** is the project manager and finance provider for this project. **S. Makchuchit** examined and analyzed the results of anti-inflammatory activity. **W. Pipatrattanaseree** verified the analytical chromatographic conditions and HPLC methodology. **N. M. Davies** provided technical scientific input, helped write the initial manuscript, and revised the manuscript.

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