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Original Article

In vitro and *in vivo* evaluation of anti-inflammatory activities of ethanol extract from Lom-Am-Ma-Pruek remedy for pain relief

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Abstract

Background and purpose: Lom-Am-Ma-Pruek (LAMP) remedy has been used in Thai traditional medicine to relieve pain associated with the inflammatory process. The anti-inflammatory activity and bioactivity of LAMP in an animal model have not been previously investigated. We evaluated the *in-vitro* and *in-vivo* anti-inflammatory activity of LAMP ethanol extract.

Experimental approach: The anti-inflammatory activity of LAMP and its plant ingredients were investigated on lipopolysaccharide-stimulated NO, PGE_2, and TNF-α release from RAW264.7 cells. Furthermore, the stability of LAMP under biological and chemical accelerated conditions was evaluated using the Griess reaction assay and HPLC. Lastly, rat models with ethyl phenylpropionate (EPP)-induced ear edema and carrageenan-induced paw edema were utilized to assess anti-inflammatory activity.

Findings/Results: LAMP possessed potent inhibitory effects on NO, PGE_2, and TNF-α production with IC_{50} values of 24.90 ± 0.86, 4.77 ± 0.03, and 35.01 ± 2.61 µg/mL, respectively. In addition, LAMP extract demonstrated stable biological activity, anti-inflammatory effects, and phytochemical content stability under stress conditions. Additionally, 0.5%, 1%, and 2% w/v LAMP significantly inhibited EPP-induced rat ear edema over time equivalent to 5% w/v phenylbutazone. LAMP at 180, 375, and 750 mg/kg also considerably reduced carrageenan-induced rat paw edema 2 h after carrageenan administration compared to phenylbutazone at 250 mg/kg.

Conclusion and implications: LAMP has anti-inflammatory activity by inhibiting PGE_2 formation. These findings are consistent with the efficacy and traditional use of the LAMP remedy in treating inflammatory diseases.

Keywords: Anti-inflammatory; Edema; Lom-Am-Ma-Pruek remedy; RAW 264.7; Stability.

INTRODUCTION

Musculoskeletal disorders have common symptoms including pain, stiffness, weakness, and decreased range of movement (1,2). Moreover, many musculoskeletal disorders demonstrate inflammatory processes that may lead to pain, swelling, redness, and heat in the inflamed area (3). Normally, inflammatory processes develop through the production of pro-inflammatory agents, such as interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and prostaglandin E_2 (PGE_2) (4). In Thailand, the incidence rate of musculoskeletal conditions is 19.90% which is more common in females than males (5). Acute and chronic musculoskeletal pain is a common affliction that requires ongoing treatment.

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Treatment of musculoskeletal diseases using pharmacotherapy reduces pain and the inflammatory process. In addition, there are other non-pharmacological treatments such as physical therapy and surgery.

Lom-Am-Ma-Pruek (LAMP) remedy is composed of ten plants: *Zingiber cassumunar* Roxb., *Curcuma zedoaria* (Berg) Roscoe., *Alpinia galanga* (L.) Willd., *Allium sativum* Linn., *Plumbago indica* Linn., *Piper nigrum* Linn. (white pepper), *Myristica fragrans* Houtt. both aril and seed, *Cleome viscosa* Linn., *Crateva adansonii* DC., *Crateva religiosa* G. Forst., *Erythrina variegate*, and two natural chemicals including sodium chloride (NaCl) and camphor. The LAMP remedy has been listed in the Thailand National List of Essential Medicines since 2013 for treating muscle pain and numbness (6,7). Thai traditional medicine practitioners believe that spicy herbs such as LAMP can reduce pain by reducing edema in the inflammatory area. Investigation and confirmation that LAMP and its constituents have anti-inflammatory activity for treating muscle pain is required. Thus, this research aimed to investigate the anti-inflammatory effects of LAMP and extracts of each component plant that have been used to reduce inflammation. In this study, the Griess reagent was used to measure the anti-inflammatory activity by inhibitory effects of all extracts on nitric oxide (NO) production activated by lipopolysaccharide (LPS) in RAW 264.7 cell lines. The inhibitory activity of TNF-α and PGE2 that participate in inflammation in many organs was also investigated to determine anti-inflammatory activity. Moreover, the *in vivo* anti-inflammatory effect of the ethanolic extract of LAMP was evaluated. The findings of this research are pivotal in establishing a scientific rationale for applying traditional Thai medicine in managing inflammation and analgesia associated with musculoskeletal diseases.

MATERIAL AND METHODS

Plant material

The LAMP remedy is a traditional Thai herbal formula used in various treatments. It typically includes a combination of plants. Each plant ingredient and LAMP remedy was macerated with 95% ethanol for 3 days. The maceration was repeated two times (a total three times) and dried using an evaporator (Buchi, Switzerland). The w/w extraction yield of *M. fragrans* (Mace), *M. fragrans* (Nutmeg), *A. galanga*, *C. zedoaria*, *P. indica*, *P. nigrum*, *Z. cassumunar*, *C. viscosa*, *A. sativum*, *E. variegate*, *C. adansonii*, *C. religiosa*, and LAMP remedy were 8.70, 7.17, 8.53, 7.39, 6.75, 4.47, 3.64, 3.20, 3.16, 1.95, 1.60, 1.49, and 16.11%, respectively.

The quality control methods used for the plant ingredients followed the protocols set by the Thai Herbal Pharmacopoeia (8). The parameters used in this study were loss on drying, extractive value, total ash, and acid-insoluble ash. In addition, the plant ingredients were identified and compared with an authentic herbarium collection at the Prince of Songkla University Natural History Museum (Songkhla province, Thailand).

Chemicals

Fetal bovine serum was purchased from Biochem, Germany. RPMI medium 1640, penicillin-streptomycin, trypan blue stain 0.4%, and trypsin-EDTA were purchased from Gibco, USA. Acetone, carrageenan, ethyl phenylpropionate (EPP), phenylbutazone, LPS from *E. coli* 055:B5, and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, USA. The PGE_2 enzyme immunoassay kit was purchased from Cayman Chemical, USA. Mouse TNF-alpha Quantikine ELISA kit was purchased from R&D Systems, USA. Piperine was purchased from Merck, eugenol was purchased from Fluka; myristicin was purchased from Fluka, acetonitrile, methanol, and purified water (HPLC grade) from Labscan (Bangkok, Thailand).

Cell culture

RAW264.7 macrophage cell line (ATCC TIB-71) was obtained from the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Thammasat University, Pathumthani, Thailand. Cells were grown in RPMI 1640 medium (BIOCHROMAG) supplemented with 10% heated fetal bovine serum, 50 IU/mL penicillin,

and 50 µg/mL streptomycin, and then incubated at 37 °C in a 5% CO_2 atmosphere with 95% humidity until the cells were confluent. The cells were then washed and harvested using trypsin-EDTA.

Preparation of sample solution

Ten mg of each sample extract were dissolved in 1 mL of dimethyl sulfoxide (DMSO) sterile to make a 10 mg/mL concentration. The extracts were diluted in an RPMI medium to produce the required concentrations. One hundred mL of each concentration was added to each well of the plates to obtain final concentrations of 0.01-100 µg/mL.

In vitro anti-inflammatory activity

Determination of NO levels in RAW 264.7 cell lines

RAW264.7 cells were harvested with trypsin-EDTA and diluted to a suspension in a fresh medium. The determination of the inhibitory effect of LPS-induced NO production was done in triplicate following the protocol of an established method (9). Briefly, 100 µL/well of RAW 264.7 macrophages (1×10^6 cells/well) were seeded in sterilized 96 well-plate and allowed to adhere and incubated for 24 h, at 37 °C in a humidified atmosphere containing 5% CO_2. Then the medium was removed and replaced with 100 µL/well of fresh medium containing LPS (2 ng/mL of final concentration). Various dilutions of samples were added (100 µL/well) and incubated for another 24 h. Subsequently, a 100 µL/well of supernatant was transferred into a non-sterilized 96 well-plate and added with Griess reagent (100 µL/well). The absorbance of the mixed solution was measured by a microplate reader at 570 nm. The result of the tested sample was compared with that of prednisolone, a positive control. The percentage of the inhibition of LPS-induced NO production was calculated by using the following equation, and IC_{50} values were calculated by using GraphPad Prism software (CA, USA):

$$\% \text{ of inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \quad (1)$$

where OD stands for optical density; OD control for mean of control ODs (+LPS) – mean of control ODs (−LPS); OD sample for mean of sample ODs (+LPS) – mean of sample ODs (−LPS).

After that, the medium was replaced with fresh medium containing 100 µg/mL of LPS with a test sample at various concentrations and then incubated for 24 h. NO metabolite was determined by measuring the nitrite accumulation in the supernatant using the Griess reagent (100 µL) (9). Griess reagent was added to the 96-well plates and the opacity was determined with a microplate reader at 570 nm.

MTT assay

MTT assay was used to determine the RAW 264.7 cell viability. Briefly, after 24-h incubation with test samples, MTT solution (10 µL, 5 mg/mL in PBS) was added to the wells and then incubated at 37 °C in a 5% CO_2 atmosphere with 95% humidity for 2 h. Subsequently, the medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan solution. The optical density was measured at 570 nm. The percentage of cell toxicity should be less than 30% compared with the control.

Evaluation of the inhibitory effect of LAMP extract and its ingredients on LPS-induced PGE2 in RAW 264.7 cells line

The inhibition of LPS-induced PGE_2 production was determined by using PGE_2 ELISA Kit-Monoclonal following the procedure in the manufacturer's manual. Briefly, RAW 264.7 macrophages (1×10^6 cells/well) were seeded in sterilized 96 well-plate (100 µL/well) and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2 and then the medium was removed and replaced with 100 µL/well of fresh medium containing LPS at 5 µg/mL final concentration. Various dilutions of samples were added (100 µL/well) and incubated for another 24 h. After incubation, the supernatant (50 µL/well) was transferred into 96 well-plate ELISA kit. The absorbance was measured at 412 nm using the microplate reader with a detection limit of 15 pg/mL and intra-assay and inter-assay coefficients of variation (CVs) under 15%. The result of the tested sample was compared with

that of prednisolone, the positive control. The experiment was conducted in triplicate. The percentage of the inhibition of LPS-induced PGE_2 production was calculated by using the following equation, and IC_{50} values were calculated by using GraphPad Prism software (CA, USA):

$$\% \text{ of inhibition} = \frac{\text{Mean OD sample } (+\text{LPS}) - \text{ mean OD control } (+\text{LPS})}{\text{Mean OD control } (-\text{LPS}) - \text{ mean OD control } (+\text{LPS})} \times 100 \quad (2)$$

Evaluation of the inhibitory effect of LAMP extracts on LPS-induced TNF-α release from RAW 264.7 cell line

RAW264.7 cells were seeded in 96-well plates at 1×10^5 cells/well and incubated for 24 h. Subsequently, the old medium was replaced with a fresh medium containing 5 µg/mL of LPS. Test samples at various concentrations were added and incubated at 37 °C, 5% CO_2, for 24 h. The inhibitory effects on the release of TNF-α from the supernatant were evaluated using a Mouse TNF-α Quantikine ELISA kit (CAT No. MTA00B) with detection limits of 6.23 pg/mL and intra-assay and inter-assay CVs of under 15%. Firstly, 50 µL of all samples or standards were added to appropriate wells. Secondly, 50 µL of the antibody conjugate was added to each well and the plate was sealed and incubated for 2 h at room temperature on a plate shaker. Then, the wells were washed with wash buffer. Lastly, 100 µL of substrate solution was added to each well and incubated for 30 min in the dark on a plate shaker set to 400 rpm and 100 µL of stop solution was added to each well. The 96-well plate was shaken for 1 min and then incubated for 30 min. The concentrations of TNF-α in the wells were measured with a microplate reader at 570 nm. The % inhibition of TNF-α production was calculated using the equation below, and the IC_{50} values were calculated using the GraphPad Prism software (CA, USA).

$$\% \text{ of inhibition} = \frac{\text{Mean of OD control} - \text{ mean of OD sample}}{\text{Mean of OD control}} \times 100 \quad (3)$$

The chemical components and biological stability of the LAMP extract

Ten mg of ethanolic extract was kept in accelerated condition (40 ± 2 ℃, 75 ± 5% room humidity) following the International Criteria for Harmonization (ICH) guidance. The sample was evaluated in eight time points including days 0, 15, 30, 60, 90, 120, 150, and 180. The chemical constituents of the extracts were determined using high-performance liquid chromatography (HPLC) with an ultraviolet-visible (UV-vis) detector (Spectromonitor® 4100, USA). The mobile phase consisted of a gradient elution of (water-acetonitrile: 0 min, 95:5; 5 min, 95:5; 50 min, 50:50; 60 min, 5:95; 65 min, 0:100; 65.1 min, 95:5; 70 min 95:5). The chemical markers of the LAMP were eugenol, myristicin, and piperine, followed by the validation method (7,8). Subsequently, the stability of the LAMP extract was evaluated by NO inhibitory effects on the RAW 264.7 cell line by using equation 4 provided below:

$$\% \text{ of inhibition} = \frac{\text{OD control} - \text{ OD sample}}{\text{OD control}} \times 100 \quad (4)$$

In vivo anti-inflammatory activity

Experimental animals

Sixty male Sprague-Dawley (S-D) rats (100-140 g) were purchased from Nomura Siam International Co., Ltd, Thailand. The rats were acclimatized under standard conditions (temperature at 23 ± 1 °C for 12/12-h light/dark cycle and relative humidity of 50 ± 10%) for 1 week, with free access to food and water. The experimental protocols used in this study were approved by the Animal Ethics Committee of the Faculty of Medicine, Thammasat University under approved experimental protocols (Ethic No. 024/2561).

EPP-induced ear edema in rats

This method was modified from the previous methodology to be consistent with other investigations (10,11). The anti-inflammatory activity of the LAMP extract at different concentrations (0.5%, 1%, 2% w/v) was assessed. The results were then compared with a standard anti-inflammatory drug like phenylbutazone (5% w/v) to understand dose response and to determine the minimum and maximum effective dose. By comparing the plant extract with a known non-steroidal anti-inflammatory agent like phenylbutazone, the efficacy of the plant extract against a standard treatment is benchmarked (12-16).

Thirty S-D rats, randomly allocated to five groups, six rats in each group, were treated with acetone (control group), LAMP at concentrations of 0.5%, 1%, and 2% w/v, and 5% w/v phenylbutazone, in a volume of 20 µL to the inner and outer surfaces of the right ear. Thirty min after the treatment, ear edema was induced by topical application of 5% EPP, applied in the same manner in a volume of 20 µL. The ear thickness was measured using a pocket thickness gauge (Mitutoyo, Japan) before treatment and at 30, 60, and 120 min after EPP application. The anti-inflammatory activity was calculated as the inhibition of the ear edema formation compared to the control group at each time interval.

Carrageenan-induced paw edema in rats

A previously conducted experimental protocol was modified and applied to evaluate paw edema (17,18). Thirty S-D rats were randomly divided into five groups (6 rats of each) to orally receive test substances, comprising a control group (15% w/v acacia solution), the treatment groups (180, 375, and 750 mg/kg LAMP in the vehicle), and the standard group (250 mg/kg phenylbutazone in the vehicle). After 1 h, edema was induced in the right hind paw of the rats by sub-plantar injection of 0.05 mL of a 1% carrageenan suspension in normal saline. The paw volume was measured by a plethysmometer (model 7140, UGO Basile, Italy) before treatment and at 1, 2, and 3 h after carrageenan injection.

Statistical analysis

All data were expressed as mean ± SEM or SD. Statistical significance was determined by one-way analysis of variance (ANOVA) at the 95% confidence interval. The comparisons among groups were conducted using the least significant difference (LSD) post hoc analysis. *P*-values less than 0.05 were considered significant. Statistical analysis was performed using SPSS (SPSS 13 for Windows) statistical software.

RESULTS

Identification and quality control of plant material

The plant ingredients were identified, as shown in Table 1. The LAMP remedy was evaluated for quality control standards for moisture content, total ash, acid insoluble ash, and extractive values according to the standard values set by the Thai Herbal Pharmacopoeia (THP) (9). The standard value of THP indicated that moisture content is not more than 10%, total ash is not more than 10%, and acid-insoluble ash is not more than 2% complying with the acceptable standardization values of quality of crude drug provided by the Thai Herbal Pharmacopoeia (THP). The results of the quality parameters of LAMP and its ingredients are shown in Table 2.

In vitro anti-inflammatory activity

NO inhibitory and cytotoxic effects of LAMP and its ingredients on RAW 264.7 cells

Effects of LAMP and its ingredients on the pro-inflammatory mediator, NO, in RAW 264.7 cells were measured to determine anti-inflammatory properties compared to the positive control (prednisolone). It was found that LPS stimulated the highest NO metabolite in RAW 264.7 cells at 10 ng/mL. Thus, in this study, LPS at 10 ng/mL was used to induce NO metabolites in RAW cells. The results of the inhibition activity against LPS-induced NO metabolite of LAMP and its ingredients are shown in Table 3. LAMP extract possessed a potent inhibitory effect on the NO metabolite with an IC_{50} value of 24.90 ± 0.86 µg/mL. The investigation of inhibitory effects on NO metabolites among plant ingredients showed anti-inflammatory activity in most extracts. The most effective ethanolic extract belonged *A. galanga* (L.) Willd. *C. zedoaria* (Berg) Roscoe also exhibited anti-inflammatory activity followed by *P. nigrum* Linn. and *P. indica* Linn. However, *P. indica* Linn. showed cell toxicity at 30 µg/mL. However, LAMP and its plant ingredients exhibited less anti-inflammatory activity than prednisolone.

Table 1. Plants identification and parts of plants comprising Lom-Am-Ma-Pruek remedy preparation.

Species	Family	Thai name	Plant part	Flavor	Voucher specimen number	Percentage
***Allium sativum* Linn.**	Alliaceae	Kratiem	Bulbs	Hot and spicy	SKP 006 01 19 01	7.14
***Alpinia galanga* (L.) Willd.**	Zingiberaceae	Kra	Rhizome	Hot and spicy	SKP 206 01 07 01	7.14
***Cinnamomum camphora* (L.) Presl.**	Lauraceae	Karaboon	Crystal	Hot	SKP 096 03 03 01	7.14
***Cleome viscosa* Linn.**	Cleomaceae	Pukseanphee	All of trunk	Bitter	SKP 039-1 03 22 01	7.14
***Crateva adansonii* DC.**	Capparaceae	Koombouk	Bark	Bitter and fragrant	SKP 039-1 03 01 01	7.14
***Crateva religiosa* G.Forst.**	Capparaceae	Koomnum	Bark	Bitter	SKP 039-1 03 18 01	7.14
***Curcuma zedoaria* (Berg) Roscoe.**	Zingiberaceae	Kaminaoi	Rhizome	Hot and spicy	SKP 206 03 26 01	7.14
***Erythrina variegata* Linn.**	Leguminosae	Thonglarng	Bark	Bitter	SKP 098 05 22 01	7.14
***Myristica fragrans* Houtt.**	Myristicaceae	Dokchan	Mace	Hot and fragrant	SKP 121 13 06 01	7.14
***Myristica fragrans* Houtt.**	Myristicaceae	Lokchan	Nutmeg	Hot and fragrant	SKP 121 13 06 01	7.14
***Piper nigrum* Linn.**	Piperaceae	Pikthai	Seed	Hot and spicy	SKP 146 16 14 01	7.14
***Plumbago indica* Linn.**	Plumbaginaceae	Jattamoonplengdang	Root	Hot and spicy	SKP 148 16 09 01	7.14
***Sodium chloride* (NaCl)**	-	Salt	Crystal	Salty	-	7.14
Zingiber cassumunar Roxb.	Zingiberaceae	Phai	Rhizome	Hot and astringent	SKP 206 26 03 01	7.14

Table 2. Results of quality controls of LAMP remedy and its ingredients. Data are expressed as mean ± SEM, n = 3.

Species	Moisture content (%)	Ash content (%)		Extractive values (%)	
		Total ash	Acid insoluble	Ethanolic soluble	Aqueous soluble
***Allium sativum* Linn.**	3.96 ± 0.66	3.49 ± 0.05	1.82 ± 0.07	0.90 ± 0.02	81.46 ± 1.32
***Alpinia galanga* (L.) Willd.**	9.70 ± 0.09	3.74 ± 0.00	1.87 ± 0.01	6.00 ± 0.04	15.4 ± 0.72
***Cinnamomum camphora* (L.) Presl.**	34.76 ± 0.12	1.31 ± 0.13	0.41 ± 0.03	0.04 ± 0.01	0.03 ± 0.01
***Cleome viscosa* Linn.**	8.55 ± 0.31	9.66 ± 0.12	0.24 ± 0.03	3.70 ± 0.06	14.45 ± 0.06
***Crateva adansonii* DC.**	7.43 ± 0.37	7.77 ± 0.13	0.78 ± 0.01	16.80 ± 0.17	11.4 ± 0.03
***Crateva religiosa* G.Forst.**	8.06 ± 0.30	9.74 ± 0.06	0.21 ± 0.01	1.10 ± 0.01	17.20 ± 0.06
***Curcuma zedoaria* (Berg) Roscoe.**	8.88 ± 0.10	8.14 ± 0.01	1.13 ± 0.13	7.15 ± 0.04	24.30 ± 0.12
***Erythrina variegata* Linn.**	8.47 ± 0.33	9.69 ± 0.25	1.81 ± 0.11	3.30 ± 0.03	4.55 ± 0.04
***Myristica fragrans* Houtt. (Mace)**	6.59 ± 0.29	6.27 ± 0.12	0.71 ± 0.06	7.65 ± 0.06	6.04 ± 0.04
***Myristica fragrans* Houtt. (Nutmeg)**	6.81 ± 0.27	2.48 ± 0.04	1.17 ± 0.02	18.80 ± 0.12	4.51 ± 0.02
***Piper nigrum* Linn.**	7.13 ± 0.37	2.43 ± 0.01	0.51 ± 0.01	4.55 ± 0.04	1.55 ± 0.01
***Plumbago indica* Linn.**	6.69 ± 0.34	9.02 ± 0.43	1.34 ± 0.02	8.75 ± 0.01	39.0 ± 0.04
***Zingiber cassumunar* Roxb.**	8.56 ± 0.06	5.47 ± 0.13	1.78 ± 0.01	5.35 ± 0.08	15.85 ± 0.02
LAMP	8.64 ± 0.36	7.34 ± 0.43	1.76 ± 0.05	5.30 ± 0.15	19.20 ± 1.19

LAMP, Lom-Am-Ma-Pruek.

Table 3. The percentage of inhibition and IC_{50} of LAMP extract and its ingredients on LPS-induced NO metabolite from RAW264.7 cells and cytotoxicity of plant extracts. Data are expressed as mean ± SEM, n = 3. **P* < 0.05 indicates significant differences compared to prednisolone as a positive control.

lant name	% of inhibition of NO production and (% of cytotoxicity)							IC_{50} (µg/mL)
	0.01 µg/mL	0.1 µg/mL	1 µg/mL	10 µg/mL	30 µg/mL	50 µg/mL	100 µg/mL	
Allium sativum	-	-	-	-	-	4.78 ± 2.16 (15.82 ± 6.57)	7.30 ± 4.63 (-7.24 ± 11.79)	> 100
Alpinia galanga	-0.10 ± 0.30 (-7.50 ± 6.17)	1.17 ± 2.27 (-0.55 ± 5.73)	7.13 ± 2.83 (6.42 ± 4.20)	80.76 ± 4.31 (20.34 ± 3.57)	-	-	-	6.99 ± 0.40*
Cinnamomum camphora	-	-	-	-	-	-0.44 ± 5.71 (-1.60 ± 6.57)	3.13 ± 4.63 (10.82 ± 2.08)	> 100
Cleome viscosa	-	-	-	-	-	25.22 ± 1.93 (7.56 ± 0.90)	44.17 ± 4.68 (11.84 ± 2.08)	> 100
Crateva adansonii	-	-	-	-	-	4.78 ± 2.16 (15.82 ± 6.57)	7.30 ± 4.63 (-7.24 ± 11.79)	> 100
Crateva religiosa	-	-	-	-	-	3.849 ± 1.180 (-1.71 ± 8.22)	11.433 ± 1.321 (-18.57 ± 5.86)	> 100
Curcuma zedoaria	-1.74 ± 2.24 (-16.14 ±	-1.29 ± 3.04 (-11.16 ± 3.79)	-1.82 ± 3.30 (-16.28 ± 4.23)	29.66 ± 4.07 (-2.38 ± 4.34)	97.8 ± 2.28 (4.33 ± 0.98)	-	-	14.38 ± 1.40*
Erythrina variegata	-	-	2.87 ± 0.72 (-29.34 ± 2.42)	10.75 ± 1.59 (-17.56 ± 3.29)	30.65 ± 0.44 (-15.54 ± 1.76)	53.02 ± 0.26 (7.33 ± 4.43)	85.24 ± 2.27 (16.19 ± 1.2)	47.10 ± 0.21*
***Myristica fragrans* (Mace)**	-	-	6.21 ± 3.57 (-12.95 ± 3.19)	-1.20 ± 2.71 (-11.51 ± 4.48)	5.37 ± 0.10 (-8.26 ± 5.82)	20.87 ± 2.03 (-31.92 ± 7.78)	67.36 ± 3.32 (-34.58 ± 6.93)	82.46 ± 0.59*
***Myristica fragrans* (Nutmeg)**	-	-	-8.44 ± 7.49 (-9.60 ± 3.31)	0.65 ± 5.19 (-7.52 ± 2.04)	12.56 ± 2.79 (-10.49 ± 7.58)	30.90 ± 2.11 (-12.09 ± 4.88)	80.54 ± 4.03 (-23.37 ± 7.21)	68.84 ± 1.18*
Piper nigrum	0.67 ± 3.92 (-19.24 ±	3.26 ± 3.31 (-8.70 ± 1.06)	11.76 ± 2.30 (-4.76 ± 1.53)	32.92 ± 4.50 (0.20 ± 0.59)	81.69 ± 3.37 (21.80 ± 2.98)	-	-	16.42 ± 1.19*
Plumbago indica	3.92 ± 2.54 (-11.33 ±	2.12 ± 2.66 (-18.07 ± 4.50)	-0.12 ± 2.09 (-0.13 ± 3.38)	13.07 ± 3.12 (-10.14 ± 0.36)	67.49 ± 3.61 46.65 ± 3.81	-	-	24.54 ± 1.97*
Zingiber cassumunar	-	-	1.22 ± 3.58 (-9.78 ± 4.50)	6.09 ± 4.06 (-10.38 ± 5.90)	27.99 ± 3.08 (-17.33 ± 2.86)	55.95 ± 1.51 (-18.53 ± 4.80)	91.29 ± 1.23 (-12.08 ± 5.21)	45.34 ± 1.33*
LAMP	-	-16.36 ± 4.86 (-26.70 ± 2.00)	-7.42 ± 2.48 (-22.53 ± 1.67)	16.54 ± 1.65 (-28.22 ± 5.20)	66.70 ± 4.76 (-10.71 ± 0.31)	94.04 ± 0.77 (-11.17 ± 6.50)	-	24.90 ± 0.86*
Prednisolone	-	32.35 ± 1.87 (3.97 ± 1.29)	46.11 ± 0.48 (7.44 ± 3.18)	58.45 ± 1.17 (8.71 ± 1.8)	64.86 ± 2.03 (11.79 ± 1.07)	74.03 ± 1.17 (6.57 ± 6.65)	-	1.31 ± 0.05

LAMP, Lom-Am-Ma-Pruek; LPS, lipopolysaccharide; NO, nitric oxide.

Table 4. The inhibitory effect of LAMP extract and its ingredients on LPS-induced PGE2 and their IC_{50} in RAW264.7 cells. Data are expressed as mean ± SEM, n = 3. *$P < 0.05$ indicates significant differences compared to prednisolone as a positive control.

Plants name	% of inhibition of PEG2 from RAW264.7 cells							IC_{50} (µg/mL)
	0.01 µg/mL	0.1 µg/mL	1 µg/mL	10 µg/mL	30 µg/mL	50 µg/mL	100 µg/mL	
Allium sativum	-	-	-	-	-	-	36.09 ± 2.90	> 100
Alpinia galanga	-	4.75 ± 1.9	41.66 ± 0.09	84.73 ± 3.51	81.12 ± 3.13	-	-	1.23 ± 0.01
Cinnamomum camphora	-	-	-	-	-	-	33.50 ± 1.29	> 100
Cleome viscosa	-	-	-	25.96 ± 0.34	43.29 ± 1.11	55.93 ± 0.52	81.06 ± 0.52	39.75 ± 1.46*
Crateva adansonii	-	-	-	28.51 ± 0.48	82.13 ± 6.76	86.96 ± 2.26	107.09 ± 1.45	16.78 ± 1.13*
Crateva religiosa	-	-	-	-	-	-	22.74 ± 2.18	> 100
Curcuma zedoaria	-	17.55 ± 3.38	25.77 ± 0.97	44.93 ± 1.77	74.72 ± 2.26			18.36 ± 1.05*
Erythrina variegata	-	-	-7.55 ± 0.19	54.62 ± 4.57	-	75.02 ± 2.05	81.55 ± 0.19	9.27 ± 0.72*
***Myristica fragrans* (Mace)**	-	-	47.94 ± 1.41	77.24 ± 0.30	-	103.43 ± 0.91	99.70 ± 1.21	1.57 ± 0.37
Myristica	-	-	37.97 ± 4.33	65.66 ± 4.23	-	85.40 ± 3.83	91.74 ± 1.11	4.60 ± 0.19
Piper nigrum	-	-4.99 ± 5.96	16.11 ± 0.33	94.53 ± 0.49	96.62 ± 1.61	-	-	2.95 ± 0.49
Plumbago indica	3.7 ± 2.18	7.12 ± 1.05	23.44 ± 2.38	96.49 ± 5.79	-	-	-	4.09 ± 0.32
Zingiber cassumunar	-	-	40.38 ± 1.71	78.55 ± 1.01	-	90.03 ± 2.42	102.62 ± 2.92	3.08 ± 0.34
LAMP	-	-	15.33 ± 0.08	88.44 ± 0.33	97.74 ± 0.42	96.90 ± 1.76	-	4.77 ± 0.03
Prednisolone	-3.45 ± 0.19	-3.73 ± 0.28	53.31 ± 0.09	81.83 ± 0.84	-	-	-	0.96 ± 0.01

LAMP, Lom-Am-Ma-Pruek; PGE2, prostaglandin E_2; NO, nitric oxide.

Table 5. The inhibitory effect of LAMP extract and its ingredients on LPS-induced TNF-α and their IC_{50} in RAW264.7 cells. Data are expressed as mean ± SEM, n = 3. *$P <$ 0.05 indicates significant differences compared to prednisolone as a positive control.

Plant's name	% of inhibition of TNF-α from RAW264.7 cells							IC_{50} (µg/mL)
	0.01 µg/mL	0.1 µg/mL	1 µg/mL	10 µg/mL	30 µg/mL	50 µg/mL	100 µg/mL	
Allium sativum	-	-	-	-	-	-	-42.07 ± 2.32	> 100
Alpinia galanga	-	-	-	-33.69 ± 0.7	-	-	-	> 10
Cinnamomum camphora	-	-	-	-	-	-	32.40 ± 1.85	> 100
Cleome viscosa	-	-	-	-	-	-	25.89 ± 7.45	> 100
Crateva adansonii	-	-	-	-	-	-	-35.34 ± 5.16	> 100
Crateva religiosa	-	-	-	-	-	-	-38.31 ± 7.31	> 100
Curcuma zedoaria	-	-	-	-	-38.66 ± 6.46	-	-	> 30
Erythrina variegata	-	-	-	-	-	-	19.82 ± 7.81	> 100
***Myristica fragrans* (Mace)**	-	-	-	-	-	-	30.42 ± 1.07	> 100
Myristica	-	-	-	18.73 ± 6.02	13.41 ± 3.65	51.17 ± 0.71	64.08 ± 1.46	49.36 ± 0.42*
Piper nigrum	-	-	-	-	31.49 ± 7.83	-	-	> 30
Plumbago indica	-	-	-	-	-58.38 ± 10.89	-	-	> 30
Zingiber cassumunar	-	-	-	42.01 ± 4.07	63.10 ± 3.8	72.86 ± 3.25	65.07 ± 0.80	16.90 ± 3.54*
LAMP	-	-	8.55 ± 5.79	6.20 ± 2.10	40.58 ± 4.48	71.06 ± 2.79	-	35.01 ± 2.61*
Prednisolone	-	34.16 ± 4.09	50.93 ± 3.14	70.50 ± 4.07	-	86.83 ± 2.21	-	0.95 ± 0.19

LAMP, Lom-Am-Ma-Pruek; TNF-α, tumor necrosis factor-alpha; NO, nitric oxide.

Inhibitory effect of LAMP and its ingredients on LPS-induced PGE2 in RAW 264.7 cells line

The inhibitory effects of LAMP and its plant ingredients on LPS-induced PGE2 are shown in Table 4. LAMP extract demonstrated inhibitory activity on PGE2 production induced by LPS-in RAW 264.7 cells with an IC_{50} value of 4.77 ± 0.03 µg/mL, which was not significantly different from the positive control (prednisolone). The extracts of *A. galangal, M. fragrans (Mace), M. fragrans (Nutmeg), P. nigrum, P. indica,* and *Z. cassumunar* exhibited strong anti-inflammatory activity by inhibiting LPS-stimulated PGE2 release. The observed inhibitory effect was not significantly different from that of prednisolone, which served as the positive control in this study.

Assay for inhibitory effect on LPS-induced TNF-α release from RAW 264.7 cell line

The inhibition effects on LPS-induced TNF-α release from RAW 264.7 cell line using Quantikine mouse TNF-α ELISA test kit of LAMP and its ingredients are shown in Table 5. LAMP extract possessed potent inhibitory activity against TNF-α release less than prednisolone. In addition, *Z. cassumunar* exhibited moderate activity. *M. fragrans* (nutmeg) has weak activity. All compounds and extracts evaluated exhibited no toxicity against RAW 264.7 cells at low concentrations.

The stability control of the LAMP extract

All LAMP extracts from the stability test (days 15, 30, 60, 90, 120, 150, and 180) were not significantly different in anti-inflammatory effect by inhibiting NO production release from RAW 264.7 cells compared with day 0 (Table 6). Simultaneously, the chemical contents including eugenol, myristicin, and piperine from days 0 until 180 demonstrated no significant differences except for eugenol at days 60 and 180 compared to day 0 (Table 7). The identification of marker chemicals compared with LAMP extract is shown in Fig. 1. Standard curves were: eugenol y = 53.33x-25.99; R^2= 0.99985 at 210 nm, myristicin y = 139.02x-790.75; R^2= 0.9997, at 210 nm, and piperine y = 22.93x-903.99; R^2= 0.9997 at 256 nm.

Table 6. The percentage of inhibition and IC_{50} of LAMP extract under the accelerated condition on LPS-induced NO metabolite from RAW264.7 cells and cytotoxicity. Data are presented as mean ± SEM, n = 3. **P* < 0.05 indicates significant differences compared to the respective day 0.

Sample	% of inhibition of NO production and (% of cytotoxicity)				IC_{50} (µg/mL)
	1 µg/mL	10 µg/mL	30 µg/mL	50 µg/mL	
Day 0	-7.42 ± 3.01 (-12.62 ± 4.48)	28.54 ± 4.73 (-3.18 ± 1.11)	51.7 ± 3.09 (12.02 ± 4.88)	94.04 ± 2.45 (19.42 ± 2.21)	28.16 ± 1.57
Day 15	-5.91 ± 0.99 (-1.90 ± 4.47)	30.47 ± 2.39 (3.77 ± 1.07)	50.11 ± 0.65 (10.65 ± 3.03)	87.36 ± 1.36 (26.58 ± 1.59)	29.92 ± 1.46
Day 30	-3.17 ± 5.27 (-8.01 ± 1.95)	55.10 ± 0.81 (-0.23 ± 8.31)	49.01 ± 0.98 (1.32 ± 4.72)	77.57 ± 1.84 (17.49 ± 2.26)	23.07 ± 2.44
Day 60	1.04 ± 3.12 (-21.22 ± 3.38)	35.02 ± 2.64 (-4.61 ± 2.4)	48.47 ± 1.77 (-4.01 ± 3.88)	79.63 ± 3.03 (9.56 ± 2.58)	31.73 ± 1.45
Day 90	-2.82 ± 1.51 (-6.62 ± 6.12)	30.79 ± 0.89 (-1.39 ± 5.73)	49.91 ± 2.04 (10.25 ± 1.41)	73.82 ± 1.69 (18.52 ± 4.95)	31.96 ± 1.78
Day 120	-3.82 ± 4.66 (-10.00 ± 0.10)	28.63 ± 2.41 (-13.56 ± 3.92)	56.66 ± 2.22 (10.82 ± 2.51)	82.52 ± 2.38 (20.66 ± 3.35)	30.14 ± 2.35
Day 150	-2.83 ± 3.84 (3.16 ± 5.10)	29.22 ± 2.93 (11.18 ± 3.75)	49.01 ± 1.74 (14.35 ± 1.90)	87.36 ± 0.95 (19.20 ± 3.16)	22.25 ± 1.88
Day 180	-3.71 ± 1.72 (4.05 ± 6.27)	27.79 ± 1.82 (-1.99 ± 2.11)	53.42 ± 4.55 (21.51 ± 2.78)	77.57 ± 0.11 (26.16 ± 1.93)	26.12 ± 0.30

LAMP, Lom-Am-Ma-Pruek; LPS, lipopolysaccharide; NO, nitric oxide.

Table 7. The contents of chemical markers of LAMP extract under the accelerated condition as determined by HPLC. Data are presented mean ± SD, n = 3. *P < 0.05 indicates significant differences compared to the respective day 0.

Day	Content of markers in extract (mg/g extract)		
	Eugenol	Myristicin	Piperine
0	9.12 ± 1.52	4.30 ± 1.80	26.25 ± 1.45
15	8.24 ± 1.23	4.16 ± 1.56	23.32 ± 0.88
30	7.49 ± 1.33	4.02 ± 1.25	24.61 ± 1.54
60	5.40 ± 1.10*	4.06 ± 2.09	23.50 ± 2.25
90	8.32 ± 1.19	4.09 ± 1.17	24.17 ± 1.23
120	10.61 ± 1.06	4.04 ± 0.17	24.65 ± 1.15
150	8.37 ± 1.46	4.32 ± 1.52	26.98 ± 1.02
180	5.62 ± 1.22*	3.93 ± 1.39	23.49 ± 1.27

LAMP, Lom-Am-Ma-Pruek; HPLC, high-performance liquid chromatography.

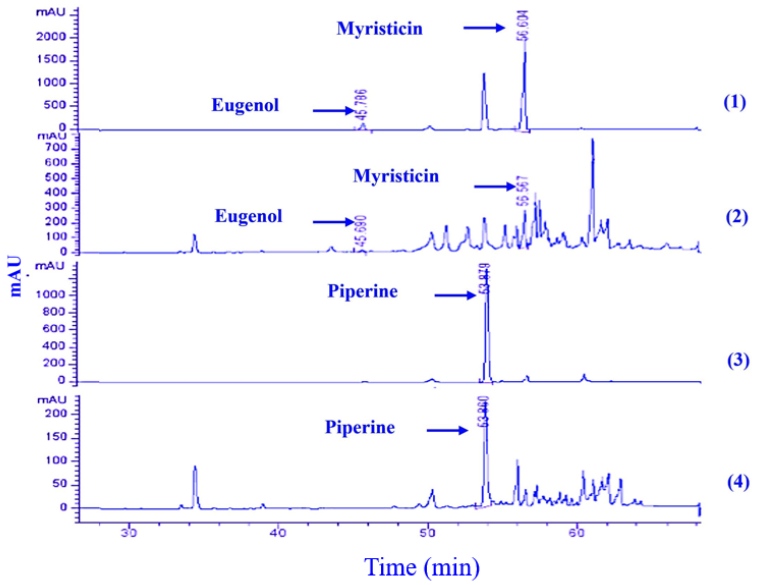

Fig. 1. The HPLC identification chromatogram of LAMP extract and the standard markers. (1) Standard markers at the wavelength of 210 nm; (2) LAMP extract evaluated at the wavelength of 210 nm; (3) standard marker at the wavelength of 256 nm; (4) LAMP extract evaluated at the wavelength of 256 nm. HPLC, High-performance liquid chromatography; LAMP, Lom-Am-Ma-Pruek.

In vivo anti-inflammatory activity

EPP-induced ear edema in rats

After induction of acute rat ear inflammation with EPP, the change in ear thickness was measured at 30, 60, and 120 min. Phenylbutazone and the LAMP extract at 0.5%, 1.0%, and 2.0% w/v significantly reduced ear edema at all time points compared to the control group. Therefore, the LAMP extract could decrease edema from an inflammatory process (Table 8).

Carrageenan-induced paw edema in rats

The sub-plantar injection of carrageenan in rats showed a time-dependent increase in paw edema. The maximum increase in edema was reached at 3 h after carrageenan administration in the control group. Phenylbutazone and LAMP (180 mg/kg) significantly inhibited the rat paw edema compared with the control group at 2 and 3 h after carrageenan administration. Administration of LAMP at 375 and 750 mg/kg also significantly reduced paw edema 2 h after carrageenan administration (Table 9).

Table 8. Inhibitory effect of LAMP on EPP-induced ear edema. Values represent mean ± SEM for each group (n = 6). * $P < 0.05$ Indicates significant differences compared to the respective control group at each time point.

Groups	Ear thickness (μm)			Edema inhibition (%)		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	13.0 ± 1.1	13.2 ± 0.9	9.6 ± 1.2	-	-	-
5% w/v phenylbutazone	9.2 ± 0.2*	9.4 ± 0.2*	8.0 ± 1.0	29.3	28.8	16.7
0.5% w/v LAMP	8.2 ± 0.7*	8.6 ± 0.9*	5.6 ± 0.8*	36.9	34.9	41.7
1% w/v LAMP	7.5 ± 0.8*	10.5 ± 1.0*	6.5 ± 0.9*	42.3	20.5	32.3
2% w/v LAMP	6.0 ± 1.2*	9.0 ± 1.3*	5.6 ± 1.3*	53.9	31.8	41.7

LAMP, Lom-Am-Ma-Pruek; EPP, ethyl phenylpropiolate.

Table 9. Inhibitory effect of LAMP on carrageenan-induced rat paw edema. Values represent mean ± SEM for each group (n = 6). * $P < 0.05$ Indicates significant differences compared to the respective control group at each time point.

Groups	Paw edema volume (mL)			Edema inhibition (%)		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	0.32 ± 0.02	0.51 ± 0.05	0.61 ± 0.03	-	-	-
Phenylbutazone (250 mg/kg)	0.31 ± 0.01	0.35 ± 0.02*	0.38 ± 0.04*	8.8	31.4	37.7
LAMP (180 mg/kg)	0.30 ± 0.05	0.30 ± 0.03*	0.43 ± 0.02*	11.8	41.2	29.5
LAMP (375 mg/kg)	0.42 ± 0.05	0.34 ± 0.05*	0.57 ± 0.04	-23.5	33.3	6.6
LAMP (750 mg/kg)	0.31 ± 0.02	0.32 ± 0.04*	0.56 ± 0.04	8.8	37.3	8.2

LAMP, Lom-Am-Ma-Pruek.

DISCUSSION

The LAMP remedy and plant ingredients demonstrated anti-inflammatory activity as measured by NO, PGE_2, and TNF-α activities. The excellent bioactivities in the LAMP remedy were derived from *A. galanga* (L.) Willd., *C. zedoaria* (Berg) Roscoe., *P. nigrum* Linn., *P. indica* Linn., and *Z. cassumunar* Roxb. The plant ingredient's bioactivity was similar to a previous study where *A. galanga* (L.) Willd ethanolic extract demonstrated inhibition of carrageenan-induced, 5-hydroxytryptamine (5-HT)-induced, and bradykinin-induced anti-inflammatory in rats of 32.22%, 37.70%, and 35.21%, respectively (19). The active compounds of *A. galanga* (L.) Willd. are 1′S-1′-acetoxy chavicol acetate, 1′S-1′-acetoxy eugenol acetate, and p-hydroxy cinnamaldehyde. The 1′S-1′-acetoxy chavicol acetate and 1′S-1′-acetoxy eugenol acetate exhibit cytokine inflammatory mediator inhibition of TNF-α and IL-4 production. (20,21). Additionally, p-hydroxy cinnamaldehyde can be a therapeutic agent for the treatment of osteoarthritis (22). *C. zedoaria* (Berg) Roscoe. has also been reported to have anti-inflammatory activity. The methanolic extract contains sesquiterpene, furanodiene and furanodienone. These compounds can decrease inflammation in mouse ears induced by 12-O-tetradecanoylphorbol acetate (75% and 53%, respectively) (23). The essential oil derived from *C. zedoaria* (Berg) Roscoe demonstrated notable therapeutic potential, particularly in the management of gynecologic inflammation, as evidenced by its application within traditional Chinese medicine (24), and may also contribute to the reduction of gingival inflammation (25). *P. nigrum* Linn. has piperine as a chemical marker for anti-inflammatory activity. Piperine at 10 and 15 mg/kg revealed anti-inflammatory and analgesic effects using carrageenan-induced paw edema, tail immersion, analgesia meter, hot plate, and writhing methods (26). *P. indica* Linn. has also been used for anti-inflammatory properties in folk medicine. Plumbagin exhibited anti-inflammatory and analgesic effects at doses ranging from 5 to 20 mg/kg, which suppressed carrageenan paw edema in rats (27). *Z. cassumunar* Roxb. has been investigated and the oral administration of a methanol extract (3 g/kg) exhibited anti-inflammatory activity against edema and reduced the number of writhes induced by acetic acid (28). The rhizomes of *Z. cassumunar* Roxb. were used as an anti-asthmatic drug in Thai traditional medicine

and topical application (29). The (E)-1-(3,4-dimethoxy phenyl) butadiene is a marker chemical in *Z. cassumunar* Roxb., it can inhibit sulfated glycosaminoglycans and hyaluronic acid release from cartilage, which relieves pain and inflammation in osteoarthritis and rheumatoid arthritis (30).

The stability of the LAMP extract showed the biological and chemical fingerprint stability under accelerated conditions. These experiments can assume the efficacy of LAMP extract for two years following ICH, 2003. The chemical marker from plant ingredients is piperine. A previously conducted study on piperine elucidated its stable anti-inflammatory activity by applying the Sa-Has-Ta-Ra remedy methodology (31). Conversely, piperine exhibited degradation upon exposure to acid, base, and oxidative stress (32) like eugenol and myristicin (33). Therefore, the LAMP extract preservation must be protected in a suitable container.

The *in-vivo* findings of the current study revealed that LAMP extracts at the lowest oral dose (180 mg/kg) caused optimal paw edema inhibition compared with the intermediate (375 mg/kg) and the highest dose (750 mg/kg) at 2 h. Compared with phenylbutazone, all doses of LAMP extract demonstrated an inhibition rate of > 30% at 2 h. However, the inhibition rate of all doses of LAMP extract at 3 h was decreased by >20% except for the lowest dose. Hence, the lowest dose was suitable for development in future investigations for oral administration. As a result, LAMP demonstrated significant anti-PGE2 production in cell culture compared to anti-NO and TNF-α production. PGE2 is an inflammatory cytokine involved in acute inflammation (34). The carrageenan-inducing paw edema model has two periods: the early phase (0-1 h) releases anti-inflammatory mediators (histamine, serotonin, bradykinin, and prostaglandins). The second phase releases various cytokines, including IL-1β, IL-6, IL-10, and TNF-α (35). The LAMP ethanolic extract could better inhibit PGE2 in the acute phase than TNF-α.

Therefore, all doses of LAMP extracted after oral administration at 2 h demonstrated more inhibition than at 3 h. Meanwhile, the topical administration of LAMP inhibited the EPP-induced ear edema dose independently. The highest inhibition rate was at 1 h. EPP-ear edema is different from the carrageenan-inducing model method. The mechanism of EPP-induced edema increases vascular permeability and releases a variety of inflammatory mediators like the acute early phase of carrageenan-inducing paw edema (10). Therefore, the LAMP extract in both methodologies was excellent for anti-cytokine mediator release in the acute phase.

Overall, the plant's ingredients in the LAMP remedy increased synergistically in bioactivity. Thai traditional remedies may demonstrate synergistic effects. For instance, Sa-Has-Ta-Ra and Ben-Ja-Kul remedies result in excellent activity from the plant's ingredients coordinated in the remedy (31,36). The concept of Herbal Formulation in Thai Traditional Medicine (HFTTM) consists of primary effect drugs, supporting effect drugs, maintenance effect drugs, and medicine flavoring following medicinal properties from the herb flavor (37). The plant's ingredients in LAMP (*A. galanga* (L.) Willd., *C. zedoaria* (Berg) Roscoe., *P. nigrum* Linn., *P. indica* Linn., and Z. cassumunar Roxb.) demonstrated predominant anti-inflammatory efficacy with a spicy flavor. The mechanism of herbal flavor contributes to Thai traditional pharmacology. The spicy flavor in Thai traditional treatment theory was used to treat the imbalance of wind and fire elements such as pain, obstructive airway, flatulence, and tiredness. The Thai traditional medicine theory is like Ayurvedic Pharmacology. Ayurvedic Pharmacology calls the gustatory sense after eating “Rasa” (taste of herbal remedy). The spicy flavor is comparable to “Katu” (pungent). The Katu interaction increases the body's wind and fire elements. In addition, the Katu has dealt with vascular system obstructions. (38). Additionally, the herbal remedies including Sahastara remedy (39) and Benjakul remedy (40) have ingredients similar to the LAMP remedy. The results of Sahastara remedy (39) and Benjakul remedy (40) demonstrated anti-inflammatory activity in knee osteoarthritis. Therefore, anti-inflammatory activity both *in vitro* and *in vivo* relates to Thai traditional treatment and herbal formulation. In the future, given the anti-

inflammatory effect demonstrated both *in vitro* and *in vivo* in two animal models, the LAMP extract should be examined for safety and efficacy in clinical trials or pain relief compared to conventional anti-inflammatory treatments.

CONCLUSION

LAMP inhibited inflammation through PGE2, TNF-α, and NO inhibition. LAMP extract showed acute anti-inflammatory activities through the inhibition of EPP-induced ear edema and carrageenan-induced paw edema in rats. The effect of LAMP may be mediated through PGE2. Based on these results, LAMP represents a potential Thai drug formulation to be further evaluated for treating inflammatory diseases.

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Conflict of interest statement

The authors confirmed no conflict of interest in this study.

Authors' contributions

A. Itharat conceived and supervised the project, obtained funding, and wrote the manuscript; P. Thongmee, K. Piwngam, J. Inprasit, and S. Makchuchit carried out the experiments and wrote the manuscript; Kuropakornpong carried out the experiments and wrote and revised the manuscript; N.M. Davies wrote and revised the manuscript. All authors read and approved the finalized article.

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