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Anti-inflammatory Activity of Extracts from Thai Herbal Compress Ball and Its Plant Ingredients

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ABSTRACT

Look Phra Ang Kob (LPAK), one of Phra Osod Phra Narai remedies, is the Thai traditional herbal compress ball which has long been used to relieve local muscle pain such as office syndrome. This compress ball consists of six ingredients including *Tamarindus indica* L., *Crinum asiaticum* L., *Zingiber cassumunar* Roxb., *Cinnamomum verum* J. Presl.Z, *Nigella sativa* L., and sodium chloride. The objective of this study was to investigate the anti-inflammatory effects of the LPAK formula used in a hospital, for each ingredient and for the modified LPAK formulae. The ethanolic extract and decoction were prepared and tested for the inhibition of nitric oxide production by LPS activated RAW264.7 cell line. The results shown that the best formula was the ethanolic extract of LPAK formula 3 (IC_{50} value 48.14). The water extract of *Crinum asiaticum* L. exhibited excellent inhibitory effect on NO production with IC_{50} value of 9.45 ± 2.17 µg/mL and the ethanolic extracts of *Crinum asiaticum* L. and *Zingiber cassumunar* Roxb. also showed good inhibitory effect on NO production; IC_{50} value of 17.73±0.75 and 14.80±2.00 µg/mL, respectively. The results of this study supported the traditional use of LPAK and some plant ingredients.

Keywords: Look Phra Ang Kob; Phra Osod Phra Narai scripture; Thai herbal compress ball; Anti-inflammatory; Nitric Oxide

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1. Introduction

The Look Phra Ang Kob (LPAK) recipe was listed as the 57th recipe in the Phra Osod Phra Narai scripture initiated by King Narai Maharaj in the Ayuthaya Period (1893-2306 B.E.). This book contains hundreds of Thai herbal and Traditional Thai medicine formulae. These recipes were particularly used in the past to treat the king or the members of royal family [1]. LPAK was a kind of herbal compress ball used to relieve muscle strain or sprain. This remedy consists of five herbs and one inorganic material namely *Tamarindus indica* L.(TI), *Crinum asiaticum* L. (CA) , *Zingiber cassumunar* Roxb. (ZC), *Cinnamomum verum* J. Presl. Z (CV), *Nigella sativa* L. (NS) and sodium chloride (NaCl) at the ratio of 16: 8: 4: 2: 1: 1, respectively [2]. At present, these LPAK recipes are being used to treat patients with muscle pain in the King Narai Hospital, Lopburi province.

The National List of Essential Medicines of Thailand also included a recipe for an herbal compress ball called Ya Pra Kob (YPK) that comprises *Z. cassumunar* Roxb. (50g) , *T. indica* L. (30g) , *Citrus hystrix* DC. (20g), *Curcuma longa* L. (10g), *Cymbopgon citratus* (DC.) Stapf. (10g) , *Acacia concinna* (Willd.) D.C. (10g) , Camphor (30 g), and sodium chloride (15g). The indication is to relieve local muscle pain [3].

Myofascial pain syndrome (MPS) is a common painful muscle disorder caused by myofascial trigger points (MTrPs), one of a musculoskeletal disorders (MSDs) [4]. Apparently, nitric oxide (NO) is involved in many physiological processes and plays a complex and diverse role in the modulation of inflammation process and pain [5]. When the injury occurs, macrophage acts as the first line of defense against any invading agent and releases cellular signaling molecules and various pro-inflammatory cytokines or mediators such as nitric oxide. Kosh and coworkers [6] demonstrated that there was significant increase in plasma levels of NO in chronic pain, especially for MPS patients in comparison with healthy controls.

The usual treatments of muscle pain are non-pharmacological treatments, complementary therapies and pharmacological interventions (eg. analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) , corticosteroid injections) [7].

However, there was no report on the anti-inflammatory activities of either LPAK or YPK compress balls. Previous studies reported that TI exerted anti-inflammation by inhibiting the production of PG and NO. [8-9]. The leaf ethanolic extract of CA showed an inhibitory activity of NO production with an IC_{50} value of 58. 5 μg/ mL [10]. The ethanolic extract of ZC rhizome exhibited a potent inhibitory effect on NO release with IC_{50} value of 4.35±0.00 mg/mL [11]. The 90% ethanol extract of CV bark at 50 μg/ mL inhibited 65.1% of NO production [12]. Thymoquinone, the active ingredient of NS, at a dose of 25 μM strongly inhibited the production of nitric oxide [13].

The objective of this study was to investigate the anti-inflammatory effects of 3 LPAK formulae and each ingredient in comparison with YPK. The results can be used to support the use of LPAK compress ball for the treatment of muscle pain and other MSDs in this hospital.

2. Materials and Methods

2.1 Plant materials

The leaves of *T. indica* L., the leaves of *C. asiaticum* L., and the rhizome of *Z. cassumunar* Roxb. were collected from Lopburi province, Thailand, in 2017. Seeds of *N.sativa* L. and bark of *C. verum* J. Presl. were bought from herbal shops in Bangkok, Thailand. The natural salt (NaCl) was bought from Lopburi province, Thailand.

Ya pra kob (YPK, used as control) is a Thai herbal compress ball remedy in the National List of Essential Medicines of

Thailand. The total content of 130 g consists of the rhizome of *Z. cassumunar* Roxb. (50g), the leave of *T. indica* L. (30g), the peels of *C. hystrix* DC. (20g), the rhizome of *C. longa* L. (10g), the aerial part of *C. citratus* (DC.) Stapf. (10g), the leaves of *A. concinna* (Willd.) D. C. (10g), camphor (30g), and sodium chloride (15g) (Vejpongosot, Thailand). The verification of the plant identities was done by an expert at the Herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Science, Prince of Songkhla University, Songkhla, Thailand (Table 1, Table 2).

Table 1. Plant ingredients in LPAK compress ball.

Scientific name	Family	Common names	Thai name	Voucher specimen number	Code
Cinnamomum verum J. Presl.	LAURACEAE	Cinnamon	Ob choie ted	SKP 296 03 12 01	CV
Crinum asiaticum L.	AMARYLLIDACEAE	Poison bulb	Plub pleung	SKP 189 48 02 01	CA
Nigella sativa L.	RANUNCULACEAE	Black Cumin	Tian dam	SKP 199 03 03 01	NS
Tamarindus indica L.	LEGUMINOSAE	Tamarind	Ma kham	SKP 200 56 04 01	TI
Zingiber cassumunar Roxb.	ZINGIBERACEAE	Bengal ginger	Phlai	SKP 206 26 03 01	ZC
Sodium Chloride (NaCl)	-	Salt	Kluea	-	NaCl

Table 2. Plant ingredients in YPK compress ball.

Scientific name	Family	Common names	Thai name	Voucher specimen number
Acacia concinna (Willd.) D.C.	LEGUMINOSAE	Soap pod	Sompoi	SKP 098 01 03 01
Citrus hystrix DC.	RUTACEAE	Kaffir lime	Ma krud	SKP 166 03 08 01
Curcuma longa L.	ZINGIBERACEAE	Turmeric	Kaminchan	SKP 206 03 12 01
Cymbopgon citratus (DC.) Stapf.	GRAMINEAE	Lemongrass	Takrai baan	SKP 081 03 03 01
Tamarindus indica L.	LEGUMINOSAE	Tamarind	Ma kham	SKP 200 56 04 01
Zingiber cassumunar Roxb.	ZINGIBERACEAE	Bengal ginger	Phlai	SKP 206 26 03 01
Sodium Chloride (NaCl)	-	Salt	Kluea	-
1,7,7-Trimethylbicyclo [2.2.1] heptan-2-one	-	Camphor	Karaboon	-

2.2 Preparation of the extract

All plants were cleaned, sliced and dried at 50°C for 72 hrs. in an oven and ground to coarse powder. Three formulae of LPAK were prepared according to Table 3. LPAK formula 1 contained fresh plant materials of *T. indica* L., *C. asiaticum* L., and *Z. cassumunar* Roxb., in LPAK formula 2 these three plant materials were used as dried powder. LPAK formula 3 contained dry material of these 3 ingredients in equal weight as fresh material in LPAK formula 1.

Each plant ingredient of LPAK, the three formulae of LPAK and YPK were extracted by maceration with 95% ethanol for 3 days, filtered and the marc was re-macerated twice. The combined filtrates were evaporated by rotary evaporator to dryness. The decoction of each plant ingredient of LPAK and the three LPAK formulae were prepared by boiling with water until one-third remained. The water extracts were then dried in a lyophilizer. All extracts were kept at -20°C until required.

Table 3. The three formulae of LPAK remedies.

Scientific name	Part of use	Flavor	Thai traditional use	Content		
				LPAK Formula 1	LPAK Formula 2	LPAK Formula 3
Tamarindus indica L.	Leave	Sour, Astringent	Analgesic	50 g (fresh)	46.85 g	50 g
Crinum asiaticum L.	Leave	Astringent, Bitter	Muscle pain, postpartum care	25 g (fresh)	7.36 g	25 g
Zingiber cassumunar Roxb.	Rhizome	Hot, Astringent	Muscle pain, Gynecology	12.5 g (fresh)	4.36 g	12.5 g
Cinnamomum verum J. Presl.	Bark	Hot, sweet, spicy	Astringent, Carminative	6.25 g	6.25 g	6.25 g
Nigella sativa L.	Seed	Bitter, Hot, Spicy	Carminative, Antipyretic	3.125 g	3.125 g	3.125 g
Sodium Chloride (NaCl)	Crystal	Salty	Gynecology	3.125 g	3.125 g	3.125 g

2.3 Chemicals & Reagents

RAW 264.7 murine macrophage leukemia cell line was obtained from American Type Culture Collection (ATCC TIB-71); ethanol 95%, chemical grade from (Hong Huat Company Limited, Thailand); distilled water Milli-Q ≥ 18 Mega Ohm from (Milford, USA); dimethyl sulfoxide [(CH3)2SO] (DMSO) (RCI Labscan, Thailand) for quality control of plant materials. Chemicals purchased for cell culture and bioactivity tests included Fetal bovine serum (FBS) from Biochem, Germany; penicillin-streptomycin (P/S), Dulbecco's Modified Eagle medium (DMEM), tryphan blue stain 0. 4%, and trypsin-EDTA from Gibco (USA); N-(1-Naphthy) ethylene-diamine dihydrochloride, phosphoric acid solution, thiazolyl blue tetrazolium bromide (MTT), and sulfanylamide from Sigma (USA); phosphate buffered saline (PBS) from Amresco (USA); sodium Bicarbonate (NaHCO3) from BHD (England); sodium hydroxide (NaOH) from Univar (Australia); and lipopolysaccharide from E. coli 055: B5 (LPS) from Sigma-Aldrich, USA.

2. 4 Method for the evaluation of anti-inflammatory: Inhibitory effect on nitric oxide production

2.4.1 Determination of Anti-inflammatory activity by inhibition of nitric oxide production in RAW264.7

Inhibitory effects on NO production were evaluated using a modified method [11]. RAW264.7 cells (1×10^6 cells/well) were cultured in Dulbecco's Modified Eagle medium (DMEM) (100 µL/well) with added supplements; 10% heated fetal bovine serum, 50 IU/mL penicillin and 50 µg/mL streptomycin. Conditions for growth maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity. After 24 hrs.

incubation, the plate was divided into 2 parts; 48 wells (part 1) were added with 200 µL of fresh medium containing test samples and the other 48 wells (part 2) were added with 200 µL of fresh medium containing 100µg/ mL of LPS together with test samples prepared by dissolving the sample in a quantity of sterile dimethylsulfoxide (DMSO) for ethanolic extracts and the water-soluble extracts were dissolved in sterile water then filtered by 0.22 µm to give 50 mg/mL concentration and incubated for 24 hrs. Then, the aliquot (100 µL) of supernatant from 96 wells was transferred to another plate and mixed with 100 µL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphtylethylenediamine dihydrochloride in distilled water). The absorbencies of the resultant solutions were measured with a microplate reader at 570 nm. The inhibition (%) was calculated by using the following equation and the IC_{50} values were determined by the Prism program (n=3).

$$Inhibition\ (\%) = \left[\frac{(\text{ODcontrol} - \text{ODsample})}{\text{ODcontrol}}\right] x\ 100$$

2.4.2 Cell viability by MTT assay

This method is done for seeking optimal concentration used for NO production study to ensure that cells are healthy for bioactivity assays and the concentrations used are not toxic to the cells. This assay determined cell viability by colorimetry using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT). The viability should be more than 70%.

After the first 24 hrs. of incubation, the 48 wells without added LPS (part 1) had 10 µL of MTT solution added (5 mg/ mL in PBS) and were incubated for 2 hours. Then, the medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formed formazan. The optical density was then read with a microplate reader at 570 nm. The inhibition (%) was calculated by using the following equation.

$$\%\ Survival = \frac{(\text{Sample O.D. x 100})}{\text{Control O.D.}}$$

2.5 Statistics analysis of NO and MTT assay

All data were the mean of three replicates. Values of different parameters were expressed as the mean ± standard error of mean. The statistical significance was calculated by one-way analysis of variance ($p < 0.05$).

3. Results and Discussion

The LPAK remedy extracts in various formulae and individual ingredients were tested for anti-inflammatory activity against LPS-induced NO production compared to prednisolone and YPK. All extracts at 100 µg/mL showed more than 70% viability by MTT ascertained that they were not toxic to cells, except CAW and prednisolone were used at 15 and 50 µg/mL, respectively (Fig. 1).

The inhibitory effect of nitic oxide assay, among individual plant ingredients indicated that the most active extract was CAW (IC_{50} values of 9.45±2.17 µg/mL), followed by ZCE, CAE, and TIE (IC_{50} values of 14.80±2.00, 17.73±0.75, and 80.24 ±1.44, respectively). Both ethanolic extracts and decoctions of CV and NS were inactive in reduction of NO production (IC_{50} value > 100 µg/mL). There were 11 samples without inhibitory effects on NO production (IC_{50} value > 100 µg/ mL) (Fig. 2). The results of this study suggested that the active ingredient of the LPAK remedy might be *Crinum asiaticum* due to its greater activity on inhibitory effects of NO production and it was the major ingredient. Moreover, lycorine which is the active compound of *Crinum asiaticum* has been reported to have anti-inflammatory inhibition of NO production. [10, 14]

The 95% ethanolic extract on LPAK formula 3 (L3E) was shown to have the best

inhibitory effect on NO production with IC_{50} value of 48. 14±3. 10 μg/mL. Other LPAK formulae did not show any activities (IC_{50} value > 100 μg/mL). The results of this study were consistent with Laokam et al study [15]. They found that the oven dried *Crinum asiaticum* leaves increased lycorine content. The LPAK formula 3 comprised dried herbal ingredients of TI, CA and ZC which were equivalent to higher amount of fresh weight than those in LPAK formula 1. The ratio of LPAK herbal ingredients in formula 1 according to Table 3 was 16:8:4:2:1:1 but the ratio of ingredients in LPAK formula 3 was 17:27:11:2:1:1 (Table 5). The fact that CA contains lycorine which has an inhibitory effect on NO production [14] was the reason for better activities of formula 3 over formula 1.

Moreover, the ethanolic extracts of L3E and YPK were comparable ($P>0.05$) in their activities. These results demonstrated that L3E which contained fewer ingredients than YPK (8 ingredients) was as effective as YPK in inhibitory effect on NO production. All the formulae including YPK show less inhibitory effect on NO production than prednisolone (IC_{50} value of 1.53±0.17 μg/mL). However, prolonged use of prednisolone could cause serious side effects.

Our study has shown that modified LPAK formula using dried material of TI, CA and ZC in equal amount of fresh materials (LPAK formula 3) gave comparable results to that of YPK. Therefore, LPAK formula 3 could be considered as a potential candidate for alternative compress ball with the advantage of being more economical due to fewer ingredients were used and proven activity. However, a clinical study should be performed to establish its efficacy.

Both LPAK and YPK share two similar ingredients but in different ratios. The LPAK recipe consists of TI: ZC ratio 4:1 while the ratio in YPK was 1:1.7; however, LPAK also have CA twice as much as ZC. This explained the comparable activity of LPAK to YPK due to high activity in CA. TI the most abundant ingredient in LPAK was included for its Thai traditional uses as relaxant and antipruritic properties [16].

This study has shown for the first time the anti-inflammatory activity of LPAK and YPK using inhibitory effect on NO production; however, other inflamematory mediators such as prostaglandin E2 (PGE_2) or pro-inflammatory cytokines such as interleukin (IL-1β, IL-6), and tumor necrosis factor (TNF)-α are also involved in the process of pathological pain [17]. Therefore, it is recommended that LPAK formula 3 and YPK should be further tested for these inflammatory mediators. The results also suggested that the water soluble part of LPAK formula 3 (L3W) was inactive; therefore, the removal of this part would increase the activity of this formula.

Table 4. Yields (%w/w) and percentage of inhibition of LPS induced NO production (IC_{50} µg/mL±SEM) of plant extracts (n=3).

Sample	Extraction	Code	%Yield	Inhibition of NO production (%) from RAW264.7cells at various concentration (µg/mL)									IC_{50} ± SEM (µg/mL)
				0.100	1.000	1.875	3.750	7.500	10	15	50	100	
LPAK formula 1	95%EtOH	L1E	8.14	-	-	-	-	-	-	-	-	61.89±3.61	>100
	Water	L1W	6.78	-	-	-	-	-	-	-	-	2.31±5.77	>100
LPAK formula 2	95%EtOH	L2E	16.40	-	-	-	-	-	-	-	-	43.63±1.13	>100
	Water	L2W	8.49	-	-	-	-	-	-	-	-	6.27±4.87	>100
LPAK formula 3	95%EtOH	L3E	20.20	-	-2.63 ±1.25	-	-	-	2.64 ±2.58	-	53.23 ±4.65	84.85±3.51	48.14±3.10
	Water	L3W	19.46	-	-	-	-	-	-	-	-	39.737±3.31	>100
T. indica L.	95%EtOH	TIE	24.38	-	-20.37 ±3.10	-	-	-	-5.28 ±2.00	-	31.77 ±1.40	61.45±0.25	80.24±1.44
	Water	TIW	22.12	-	-	-	-	-	-	-	-	1.86±3.09	>100
C. asiaticum L.	95%EtOH	CAE	13.52	-	-8.07 ±1.08	-	-	-	26.36 ±1.55	-	97.07 ±0.57	99.32±0.31	17.73±0.75
	Water	CAW	33.84	-	-	8.96 ±2.48	20.73 ±7.27	43.40 ±12.85	-	75.59 ±9.06	-	-	9.45±2.17
Z. cassumunar Roxb.	95%EtOH	ZCE	18.12	-	-14.41 ±4.95	-	-	-	32.35 ±6.91	-	92.59 ±2.16	97.25±0.33	14.80±2.00
	Water	ZCW	13.26	-	-	-	-	-	-	-	-	-6.04±1.18	>100
C. verum J. Presl.	95%EtOH	CVE	10.43	-	-	-	-	-	-	-	-	42.77±0.38	>100
	Water	CVW	8.34	-	-	-	-	-	-	-	-	-4.12±0.96	>100
N. sativa L.	95%EtOH	NSE	9.69	-	-	-	-	-	-	-	-	30.19±1.09	>100
	Water	NSW	18.59	-	-	-	-	-	-	-	-	4.80±0.63	>100
YPK formula	95%EtOH	YPK	9.986	-	-11.04 ±1.34	-	-	-	-11.48 ±0.72	-	55.70 ±9.12	95.81±1.63	48.00±4.13
Prednisolone (Positive control)	-	Pred	-	21.27 ±4.55	40.39 ±3.33	-	-	-	52.88 ±1.40	-	78.25 ±1.07	-	1.53±0.17

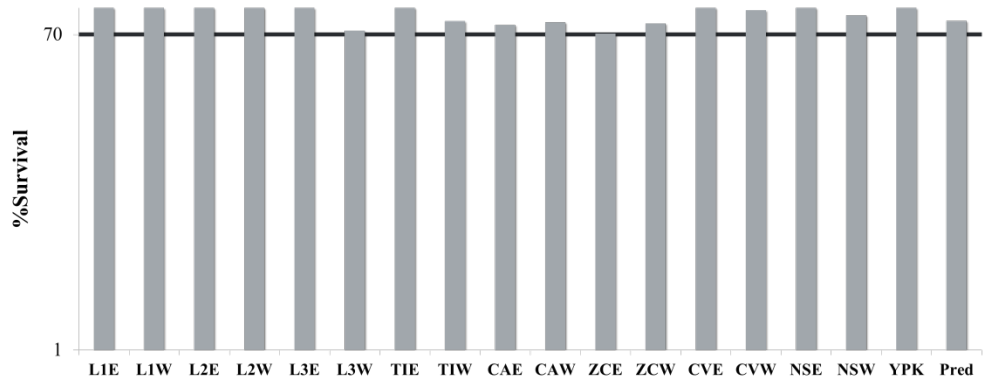

Fig. 1. Cell viability by MTT assay.
All extracts were 100 µg/mL except the water extract of *C. asiaticum* L. (CAW) and Prednisolone (Pred) were 15 and 50 µg/mL, respectively.

L1E: 95%EtOH extract of LPAK formula 1, L1W: Water extract of LPAK formula 1, L2E: 95%EtOH of LPAK formula2, L2W: Water extract of LPAK formula 2, L3E: 95%EtOH of LPAK formula 3, L3W: Water extract of LPAK formula 3, TIE: 95%EtOH extract of *T. indica* L., TIW: Water extract of *T. indica* L., CAE: 95%EtOH extract of *C. asiaticum* L. , CAW: Water extract of *C. asiaticum* L. , ZCE: 95%EtOH extract of *Z. cassumunar* Roxb. , ZCW: Water extract of *Z. cassumunar* Roxb., CVE: 95%EtOH extract of *C. verum* J. Presl., CVW: Water extract of *C. verum* J. Presl., NSE: 95%EtOH extract of *N. sativa* L., NSW: Water extract of *N. sativa* L., YPK: 95%EtOH extract of YPK formula, Pred: Prednisolone.

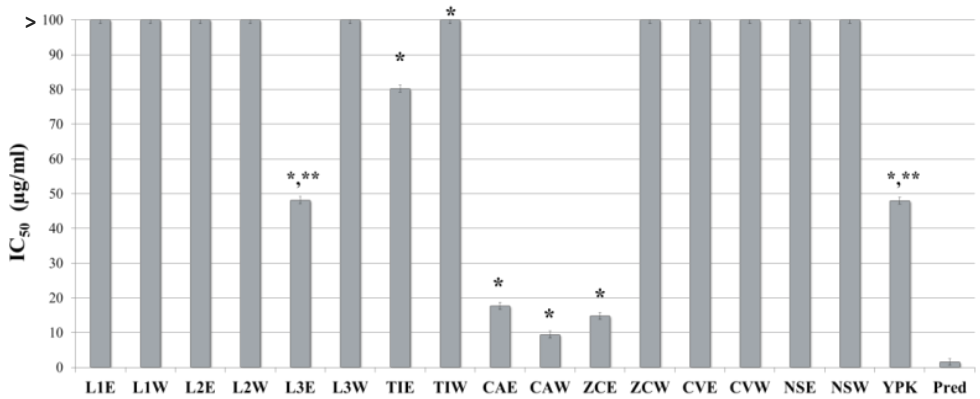

Fig. 2. The IC50 on various extracts of inhibition on LPS induced NO production from RAW264.7 cells (Mean ± SEM) (n=3).
* Significantly different ($p < 0.05$) compared with prednisolone, a positive control,
** Not significantly different ($p>0.05$).

L1E: 95%EtOH extract of LPAK formula 1, L1W: Water extract of LPAK formula 1, L2E: 95%EtOH of LPAK formula2, L2W: Water extract of LPAK formula 2, L3E: 95%EtOH of LPAK formula 3, L3W: Water extract of LPAK formula 3, TIE: 95%EtOH extract of *T. indica* L., TIW: Water extract of *T. indica* L., CAE: 95%EtOH extract of *C. asiaticum* L. , CAW: Water extract of *C. asiaticum* L. , ZCE: 95%EtOH extract of *Z. cassumunar* Roxb. , ZCW: Water extract of *Z. cassumunar* Roxb., CVE: 95%EtOH extract of *C. verum* J. Presl., CVW: Water extract of *C. verum* J. Presl., NSE: 95%EtOH extract of *N. sativa* L., NSW: Water extract of *N. sativa* L. , YPK: 95%EtOH extract of YPK formula, Pred: Prednisolone.

Table 5. Comparison of LPAK formula 1 and 3.

Scientific name	Part of use	Content			
		LPAK Formula 1	Ratio*	LPAK Formula 3	Ratio*
T. indica L.	Leave	50 g (fresh)	16	50 g (dry) = 53.36 (fresh)**	17
C. asiaticum L.	Leave	25 g (fresh)	8	25 g (dry) = 84.92 (fresh)**	27
Z. cassumunar Roxb.	Rhizome	12.5 g (fresh)	4	12.5 g (dry) = 35.84 (fresh)**	11
C. verum J. Presl.	Bark	6.25 g	2	6.25 g	2
N. sativa L.	Seed	3.125 g	1	3.125 g	1
Sodium Chloride (NaCl)	Crystal	3.125 g	1	3.125 g	1

Note: * the ratio of fresh weight, **the equivalent fresh weight calculated based on Table 3.

4. Conclusion

Our study has shown that using dried material of TI, CA and ZC with equal amounts of fresh materials in LPAK (L3E) gave comparable results to that of YPK.

However, this study is one of the mechanisms involved in inflammatory mechanism. L3E should be proven by further experiment such as anti-inflammatory activity on prostaglandin E_2 which is more related to pain, especially a clinical efficacy study should also be carried out. These data will support use of LPAK to develop products for MPS patients.

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References

[1] Sivaphongthongchai A. Wall inscription on herbal medicine and hermit exercise at Sala Ruesee Wat Matchimawas Worawihan Songkhala Province Thailand [Ph.D. thesis]. Bangkok: Chulalongkorn University; 2013.

[2] Pichiansunthorn C, Chawalit M, Jeerawong V. The describe of Osod Phra Narai textbook. Bangkok: Amarin Publishing; 2001.

[3] National List of Essential Medicines of Thailand [Internet]. [cited 2019 Jul 09]. Available from: http://kpo.moph.go.th/webkpo/tool/Thaimed2555.pdf

[4] Alvarez DJ, Rockwell PG. Trigger points: diagnosis and management. Am Fam Physician 2002;65(4):653-61.

[5] Cury Y, Picolo G, Gutierrez VP, Ferreira SH. Pain and analgesia: the dual effect of nitric oxide in the nociceptive system. Nitric oxide 2011;25(3):243-54.

[6] Koch A, Zacharowski K, Boehm O, Stevens M, Lipfert P, Von Giesen HJ, et al. Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. Inflamm Res 2006;56:32-7.

[7] Babatunde OO, Jordan JL, Van der Windt DA, Hill JC, Foster NE, Protheroe J. Effective treatment options for musculoskeletal pain in primary care: A systematic overview of current evidence. PLoS One 2017;12(6):e0178621.

[8] Bhadoriya SS, Mishra V, Raut S, Ganeshpurkar A, Jain SK. Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of *Tamarindus indica* leaves. Sci Pharm 2012;80(3):685-700.

[9] Akor ST, Wampana B, Sodipo OA. Antinociceptive and anti-Inflammatory activities of the aqueous leaf extract of *Tamarindus indica* L. in Albino Rats. J Plant Stud 2015;4(2):44-56.

[10] Kim YH, Kim KH, Han CS, Park SH, Yang HC, Lee BY, et al. Anti-inflammatory activity of *Crinum asiaticum* Linne var. japonicum extract and its application as a cosmeceutical ingredient. J Cosmet Sci 2008;59(5):419-30.

[11] Anuthakoengkun A, Itharat A. Inhibitory effect on nitric oxide production and free radical scavenging activity of Thai medicinal plants in osteoarthritic knee treatment. J Med Assoc Thai 2014;97:S116-24.

[12] Ho SC, Chang KS, Chang PW. Inhibition of neuroinflammation by cinnamon and its main components. Food Chem 2013;138(4):2275-82.

[13] Hossen MJ, Yang WS, Kim D, Aravinthan A, Kim JH, Cho JY. Thymoquinone: an IRAK1 inhibitor with *in vivo* and *in vitro* anti-inflammatory activities. Sci Rep 2017;7:42995.

[14] Kang J, Zhang Y, Cao X, Fan J, Li G, Wang Q, et al. Lycorine inhibits lipopolysaccharide-induced iNOS and COX-2 up-regulation in RAW264. 7 cells through suppressing P38 and STATs activation and increases the survival rate of mice after LPS challenge. Int Immunopharmacol 2012;12(1):249-56.

[15] Laokam S. Effect of thermal process on bioactive compounds in *Crinum asiaticum* leaves [M.Sc. thesis]. Bangkok: Chulalongkorn University; 2014.

[16] Herbal compress ball with the treatment [Internet]. [cited 2019 Aug 31]. Available from: http://www.ttmed.psu.ac.th/blog.php?p=167

[17] Zhang JM, An J. Cytokines, inflammation and pain. Int Anesthesiol Clin 2007;45(2):27-37.