Original Article

Anti-inflammatory Activities of Nanoemulsion Containing Benjakul Remedy Extract

Chunyika Thummawan*, Arunporn Itharat**, Rathapon Asasutjarit***

Abstract

Introduction: Benjakul Remedy is one of traditional Thai medicines listed in the National List of Essential Medicines of Thailand (BE 2561). It consists of five plants and is used for health balance and anti-flatulence. Previous studies showed that an ethanolic extract of Benjakul Remedy (BKE) could relieve pain in osteoarthritis knee patients via oral administration. However, it caused gastrointestinal (GI) side effects. Thus, topical nanoemulsions (NEs) containing BKE were developed for transdermal delivery of BKE to avoid the GI and systemic side effects.

Objective: The objectives of this study were 1) to develop topical nanoemulsions containing BKE (BKE-NEs) and 2) to determine physicochemical properties and anti-inflammatory activities of BKE-NEs

Method: BKE was extracted and investigated its anti-inflammatory activities by determination of inhibitory activity on nitric oxide (NO) release in RAW 264.7 cells. BKE-NEs containing various concentrations of Tween80 and BKE were prepared by using the ultrasonication technique. Physicochemical properties of BKE-NEs, i.e., droplet size, polydispersity index (PDI), zeta potential, drug release, rheology, morphology, anti-inflammatory activities and toxicity to skin cells (HaCaT cells) were also determined.

Results: BKE showed anti-inflammation activities with IC_{50} of 19.72 ± 0.72 µg/ml. BKE-NEs prepared in this study had droplet size around 25 - 900 nm with PDI of 0.2 - 1.0 and zeta potential of -0.8 to -11 mV. A representative BKE-NE (BKE3-NE-T8-x0.2) containing Tween 80 (8%w/w), BKE (3%w/w) and xanthan gum (0.2%w/w) showed the pseudoplastic flow behavior. The internal phase of BKE3-NE-T8-x0.2 had spherical shape. It could inhibit NO release from the cells with IC_{50} of 8.36 ± 1.06 µg/ml. This NE was not toxic to skin cells at all tested concentrations.

Conclusion: The optimized BKE-NE had promising physicochemical properties and showed potential for using as topical anti-inflammatory products without skin toxicity. Thus, it could be used for further studies in the animal model.

Key words: Nanoemulsion, Benjakul extract, Anti-inflammatory activity, Transdermal drug delivery.

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Introduction

The Inflammation is a part of the non-specific immune responses and pathophysiological processes. It is induced by macrophages when tissues are injured[1]. Macrophages release pro-inflammatory cytokines and the inflammatory mediators such as cyclo-oxygenase-2 enzyme (COX-2) and nitric oxide (NO). NO is one signaling molecule of the pathogenesis of inflammation. It is a pro-inflammatory mediator that is synthesized by iNOS. Nowadays, many studies indicated the relevance of the NO concentrations in the body to the incidence of many diseases[2]. Consequently, NO inhibitors present as important therapeutic agents in the management of inflammatory diseases.

Benjakul remedy is a traditional Thai medicine and listed in The National Lists of Essential Medicines (BE 2561). It consists of five plants as shown in Table 1. This remedy is used for health balance and anti-flatulence. Previous studies showed that an ethanolic extract of Benjakul Remedy (BKE) had anti-inflammatory activities through the inhibitory effect on NO release with IC_{50} of 16.60 µg/ml[2]. Furthermore, it could relieve pain in osteoarthritis knee patients via oral administration[3]. This is due to the fact that *Piper chaba* Hunt. and *Plumbago indica* Linn., components of BKE have anti-inflammatory effects similar to Non-Steroidal Anti-inflammatory Drugs (NSAIDs)[4] where they can inhibit functions of COX-2.

NSAIDs are widely used for treatment of inflammatory symptoms in Thai patients. However, some of them have suffered with the GI- and the systemic side effects such as stomach ulcers, GI bleeding including renal- and hepatic disorders from long-term use. In addition, some NSAIDs have to be imported from abroad leading to high cost of the treatment. Therefore, to overcome these problems, BKE could be considered as a proper alternative medicine for relief of inflammatory symptoms. Unfortunately, the oral BKE, sometimes, causes side effects, for example, heat, abdominal cramps, nausea, and rash[3]. Therefore, development of topical products containing BKE should be suitable for local treatment of inflammation

Nanoemulsion (NE) is an emulsion with nano-size droplets dispersed in the vehicle. It is one of promising transdermal delivery systems for the drugs, which can exhibit either local- or systemic effects[5]. NE is more effective for delivery of drug molecules to the deeper skin than the conventional emulsions. Since NE has small droplet size of the internal phase, NE is highly stable during storage and provides excellent sensory perceptions after application on skin. By the reason that NE formulations usually contain a low concentration of the emulsifier, NE rarely causes skin irritation. NE is also widely accepted by users[6]. However, there is still lack of data of NE for using as a transdermal delivery system of herbal extracts especially BKE for local treatment of inflammation. Thus, the objectives of this research were 1) to develop topical nanoemulsions containing BKE (BKE-NEs) and 2) to determine physicochemical properties and anti-inflammatory activities of BKE-NEs

Methods

Plant materials and Preparation of crude extracts

The ingredients of Benjakul remedy were purchased from local herbal drug stores in Karnchanaburi province. The specimen vouchers were referenced by the herbarium of Thai Medicinal Plants, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. BKE was prepared by the maceration technique in 95% ethanol[7].

Table 1 List of plant materials, source of plant collection and their contents in Benjakul remedy

Scientific Name	Family Name	Thai name	Part used	Voucher numbers	Source (Province)	Content (%)
Piper retrofractum Vahl., *Piper chaba* Linn. or *Piper longum* Linn.	PIPERACEAE	Di-pli	Fruits	SKP 146160301	Karnchanaburi	20
Piper sarmentosum Roxb.	PIPERACEAE	Cha-phlu	Root	SKP 146161901	Karnchanaburi	20
Piper interruptum Opiz.	PIPERACEAE	Sa-kan	Vine	SKP 146160901	Karnchanaburi	20
Plumbago indica Linn.	PLUMBAGINACEAE	Chettamun-phloeng-daeng	Root	SKP 148160901	Karnchanaburi	20
Zingiber officinale Roscoe.	ZINGIBERACEAE	Khing	Rhizome	SKP 206261501	Karnchanaburi	20

Determination of piperine content and anti-inflammatory activity of BKE

The content of piperine in BKE was determined by using the high performance liquid chromatography (HPLC) technique[8]. The chromatographic analysis was carried out using a ZORBAX® Eclipse system with XDB-C18 analytical column (4.6 x 250 mm, 5 microns). The diode array detector was evaluated at wavelength 256 nm. The sample volume of 10 µl was injected into the HPLC system. Standard solutions of piperine in acetonitrile were prepared to the final concentrations of 40, 60, 100, 200, 300 and 400 µg/ml. For sample analysis, samples containing BKE were dissolved in acetonitrile and filtered through a 0.45 µm filter membrane. Filtrates were then subjected to chemical analysis using HPLC technique.

Solubility of BKE in mixed oils

The solubility of BKE in the mixed oils consisting of oleic acid, isopropyl myristate and caprylic/capric triglyceride (1:1:1) were determined by adding BKE to the mixed oils at a ratio of 1:1. Each mixtures was shaken at 37°C for 24 hours and then centrifuged at 3,000 rpm for 15 min[9]. Ten milligrams of each supernatant was chemically analyzed by HPLC technique for the piperine content.

Preparation of NEs

NEs containing various concentrations of Tween 80 shown in Table 2. prepared by using an ultra-sonic homogenizer[10] at 20% amplitude for 5 minutes. In the cases of BKE-NEs, BKE at various concentrations was added into the oil phase prior to homogenization. To increase viscosity, xanthan gum was added to NEs. Each NE was centrifuged at 5,000 rpm for 30 min and observed for phase separation[11]. Furthermore, the piperine content in BKE-NEs was also determined by the HPLC technique.

Anti-inflammatory activity and cytotoxicity activity of BKE and BKE-NEs

Assay by determination of NO inhibitory effects in RAW 264.7 cells[12]

This assay was performed to evaluate the inhibitory effects of the samples on NO release by RAW 264.7 cells. Cells were cultured in 96-well sterile plate ($1x10^5$ cells/well) with 100 µl complete RPMI and

incubated in 5% CO_2 at 37°C overnight. Medium was then removed and replaced with Complete RPMI (100 µl/well) containing 10 ng/ml of lipopolysaccharide (LPS) or without LPS as control. Thereafter, 100 µl/well of BKE and BKE-NEs diluted with the complete medium to the final concentrations of 0.1 - 50 µg/ml were added to each well. Cells were incubated overnight. Subsequently, supernatant (100 µl) was transferred to another 96-well plate, followed by addition of Griess reagent (100 µl). The UV absorbance was measured by a spectrophotometer at wavelength 570 nm. This assay was carried out in triplicate. The inhibitory effect (%) of the samples on NO release was calculated using the following equation. IC_{50} values were calculated using the Prism program.

% Inhibition = [(C – S)/C] ×100

Control (C) : [LPS (+), sample (-)] - [LPS (-), sample (-)]

Sample (S) : [LPS (+), sample (+)] - [LPS (-), sample (+)]

The MTT assay (3-(4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H-tetrazolium bromine) was conducted to determine cytotoxicity of the samples. Plates were incubated at 37°C in 5% CO_2 incubator for 24 hours. MTT solution (10 µl, 5mg/ml in PBS) was added to each well and incubated for 2 hours. Supernatant was removed and 100 µl of isopropanol containing 0.04 M HCl added to dissolve the formazan production by cells. The optical density (OD) of formazan solution was measured by a microplate reader at wavelength 570 nm. The percentage of survival was calculated by using the following equation.

% Survival = [S/C] × 100

Control (C) : Medium, Sample (S) : Medium, sample

If the obtained values were more than 70%, they indicated that cells still survived.

Assay for MTT effects in HaCaT cells

This study was performed to determine toxicity of the samples to skin cells by using HaCaT cells which are well-known immortalized human keratinocytes. The cells were cultured in DMEM medium, and incubated at 37°C in 5% CO_2 for 24 hours. After the cells were incubated with the samples, MTT solution (10 µl, 5mg/ml in PBS) was added in each well and incubated 2 hours. Supernatant was removed and 100 µl of DMSO was added to dissolve the formazan produced by the cells. The OD was measured by a microplate reader at wavelength 570 nm. The percentage of survival was calculated by using the same equation as previously shown in the part of assay for NO inhibitory effects in RAW 264.7 cells.

Physico-chemical properties of BKE-NE

Determination of droplet size, polydispersity index (PDI), and zeta potential

Droplet size, PDI and zeta potential of BKE-NEs were analyzed by the photon correlation spectroscopy technique and the laser droppler electrophoresis technique, respectively, using a nano-size analyzer (Zetasizer Nano ZS, Malvern Instruments, Malvern, U.K.) at 25°C. Results of measurement were reported as mean ± standard deviation (SD)

***In vitro* Release Study by Franz diffusion**

Release of piperine from BKE-NE was determined by using modified Franz diffusion cells in triplicate. The optimized BKE-NE (3 g) was placed on the cellulose dialysis membrane in the donor compartment. The receptor compartment containing 10% ethanol in phosphate buffer solution (pH 7.4) to increase solubility of piperine. Throughout the study, the receiving solution was continuously stirred with a magnetic stirrer, and temperature was maintained at 37 ± 1°C. A precise amount of the receiving solution was withdrawn for determination of released piperine content by HPLC technique. The diode array detector was set at wavelength 320 nm.

Rheological properties

The rheological measurement was conducted by using a controlled-stress rheometer (Rotational Rheometer, ARES G2, TA Instruments, USA). The steady shear rate sweep test was performed at the shear rate range of 0.01-1000 s^{-1} by using the 50 mm-parallel plates geometry with a gap width of 1.00 mm.

Morphology by using transmission electron microscopy (TEM)

Morphology of the representative BKE-NE was observed by using the transmission electron microscopy technique. BKE-NE was stained with 0.5% w/v uranyl acetate solution and observed under a JEM-1220 transmission electron microscope (Japan).

Determinations of pH value of BKE-NE.

The apparent pH values of the BKE-NE were measured by a pH meter (Inolab WTW, Mexico) in triplicate at room temperature.

Results

BKE exhibited anti-inflammatory activity via inhibition of NO released by RAW 264.7 cells with the IC_{50} value of 19.72 ± 0.72 µg/ml. This value was close to IC_{50} of BKE for inhibition of NO production reported in the previous study[2]. The chemical analysis of piperine in BKE by the HPLC technique showed that one gram of BKE contained 88.89 mg of piperine. The solubility of piperine in each oil, mixed oil and surfactant (Tween 80) are shown in Figure 1. This data were useful for formulation of BKE-NEs.

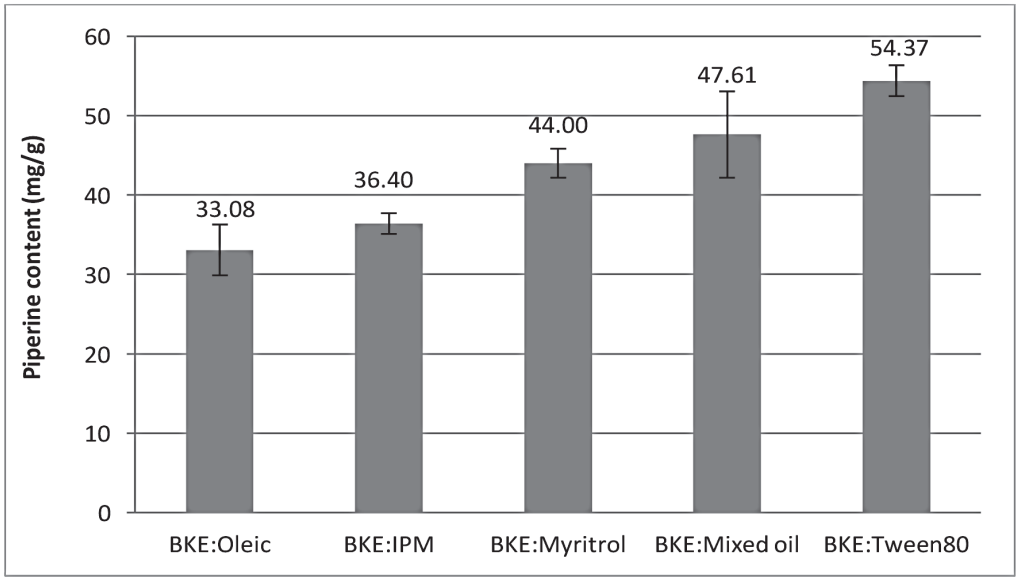

Figure 1 Piperine content in Benjakul extract in different oils at 37°C (mean ± SD, n = 3)

Droplet size, PDI and zeta potential of NE bases and BKE-NEs are shown in Table 2. It was found that all NE bases had droplet size under 200 nm with low PDI and had negative values of the zeta potential. By the reason that NE-base-T8 possessed optimum droplet size, PDI and zeta potential, it should be selected for the further studies.

After incorporation of BKE and xanthan gum, the droplet size and PDI of the nanoemulsions were obviously increased as seen in Table 2. However, NE bases and BKE-NEs prepared in this study were stable after centrifugation. Either phase separation or creaming were not found. In addition, changing in their droplet size, PDI and zeta potential were not detected during storage at ambient conditions[14].

The *in vitro* release profiles of BKE1-NE-T8-x0.2, BKE2-NE-T8-x0.2 and BKE3-NE-T8-x0.2 are shown in Figure 2. They indicated that piperine was released from BKE3-NE-T8-x0.2 faster than that of BKE1-NE-T8-x0.2 and BKE2-NE-T8-x0.2. It is important to note that release of piperine from BKE1-NE-T8-x0.2 was found after 5 hours of the experiment suggesting that release of piperine from this formulation was the slowest.

Therefore, BKE3-NE-T8-x0.2 was selected for further investigation of its physicochemical properties.

Table 2 Compositions of NE bases, BKE-NEs and their physicochemical properties

Formulation	Contents of ingredients (% w/w)				Particle size (nm)	PDI	Zeta-potential (mV)
	Mixed oil	Tween 80	BKE	Xanthan gum			
NE-base-T5	10	5	0	0	137.6 ± 0.7	0.3 ± 0.0	-4.6 ± 0.7
NE-base-T8	10	8	0	0	150.9 ± 1.8	0.2 ± 0.0	-3.8 ± 0.1
NE-base-T12	10	12	0	0	147.3 ± 0.7	0.5 ± 0.0	-3.8 ± 0.3
NE-T8-x0.2	10	8	0	0.2	567.6 ± 40.3	0.9 ± 0.2	-11.3 ± 0.6
BKE1-NE-T8-x0.2	10	8	1	0.2	497.4 ± 38.8	0.8 ± 0.2	-7.3 ± 0.9
BKE2-NE-T8-x0.2	10	8	2	0.2	537.1 ± 52.2	0.7 ± 0.2	-5.5 ± 0.1
BKE3-NE-T8-x0.2	10	8	3	0.2	587.6 ± 25.1	0.6 ± 0.9	-5.7 ± 0.2

Figure 2 (a) shows plots of cumulative amount of piperine released from BKE-NEs against time. It was found that the release profile of piperine from BKE3-NE-T8-x0.2 had a linear relationship with the R^2 of 0.995, while the plot of cumulative amount of piperine released from BKE-NEs against square root of time showed the R^2 of 0.9929 as shown in Figure 2 (b). These findings indicated that the release profile of piperine from BKE3-NE-T8-x0.2 obeyed the zero order models

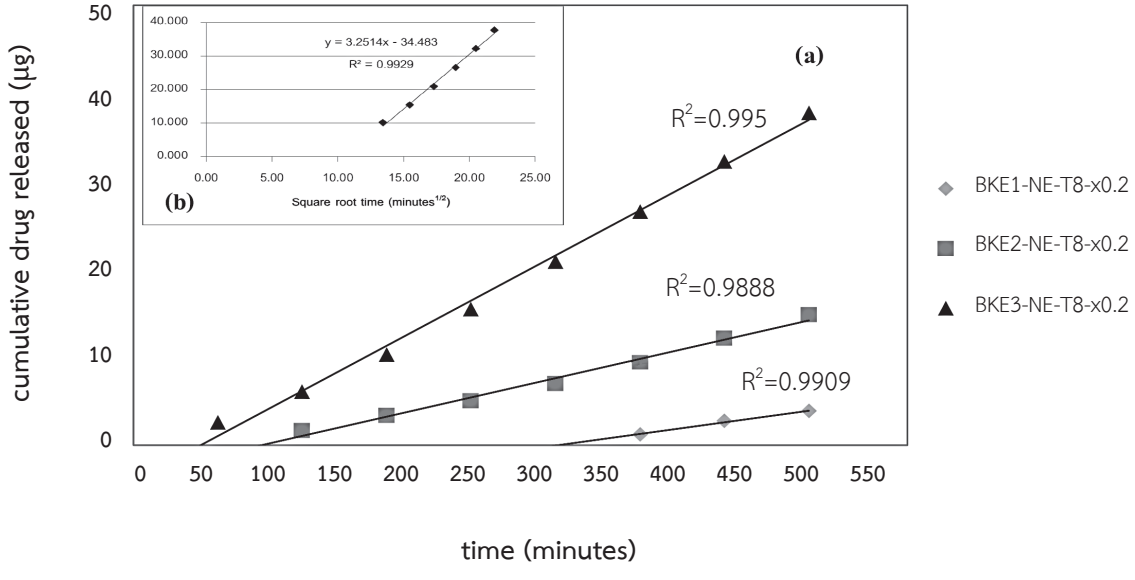

Figure 2 *In vitro* release study of BKE-NEs using Franz diffusion cells

The physicochemical properties of BKE3-NE-T8-x0.2 were shown in Table 3. It was found that BKE3-NE-T8-x0.2 contained piperine at a concentration of 0.27% w/w equivalent to 100.74% of labeled amount.

Table 3 Physiochemical properties of BKE3-NE-T8-x0.2

Parameter	Results
Structure type	O/W
pH	4.16 ± 0.09
Piperine content (g/100 g of BKE-NE)	0.269
%Labeled amount	100.74

TEM micrographs of NE-base-T8 and BKE3-NE-T8-x0.2 shown in Figure 3 (a) and (b), respectively, indicated that the morphology of the internal phase of both NEs were spherical shape with a diameter in a range of nanometer. These pictures confirm that the addition of BKE and xanthan gum led to larger droplet size of the NE.

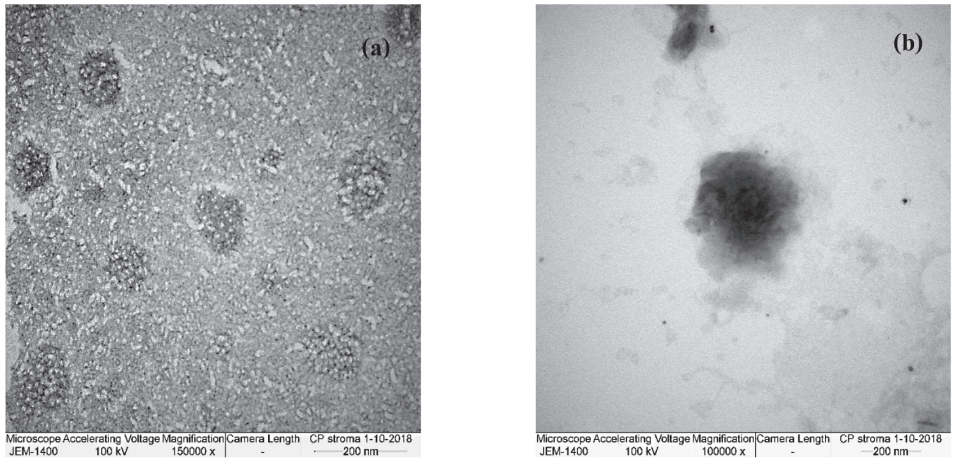

Figure 3 Morphology of (a) NE-base-T8-x0.2 and (b) BKE3-NE-T8-x0.2

The flow curve of BKE3-NE-T8-x0.2 shown in Figure 4 indicated that BKE3-NE-T8-x0.2 exhibited pseudoplastic behavior [15]. This finding was confirmed by the viscosity profile shown in Figure 5 which revealed that the viscosity of BKE3-NE-T8-x0.2 decreased with increasing shear rates.

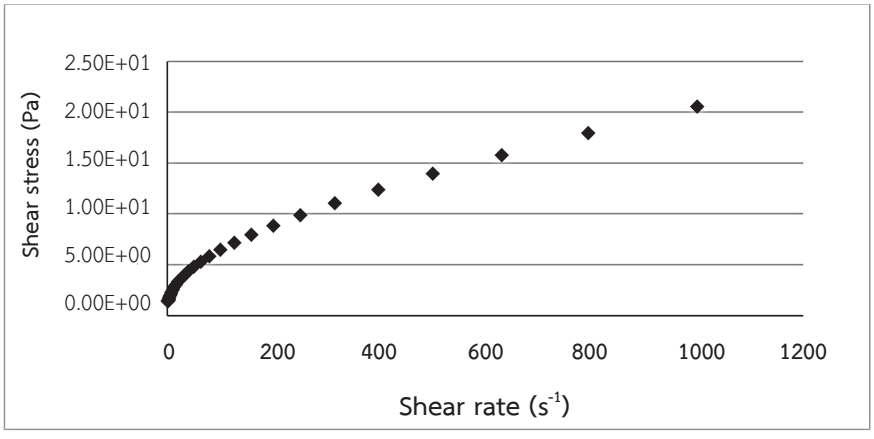

Figure 4 Flow curve of BKE3-NE-T8-x0.2

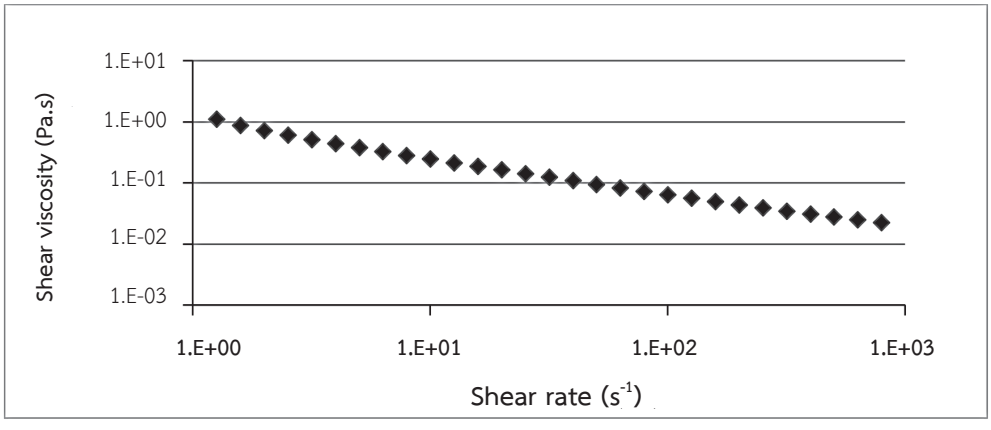

Figure 5 Logarithmic plots of viscosity against shear rates of BKE3-NE-T8-x0.2

Anti-inflammatory activity and cytotoxicity activity of BKE3-NE-T8-x0.2

Anti-inflammatory activity of BKE3-NE-T8-x0.2 is shown in Table 4. The results pointed out that BKE3-NE-T8-x0.2 possessed stronger anti-inflammatory activity than that of NE-base-T8-x0.2 and BKE, respectively. However, prednisolone, which is a positive control, was still the most potent drug for inhibition of inflammation.

The survival of HaCaT cells after being incubated with the extract and the NEs show in Table 4. Cells could survive in all mixtures consisting of the samples and the medium with the %survival more than 70%. These results suggested that BKE, NE-base-T8-x0.2 and BKE3-NE-T8-x0.2 were not toxic to the skin cells. Therefore, BKE, NE-base-T8-x0.2 and BKE3-NE-T8-x0.2 could be accepted as safe for topical application. BKE3-NE-T8-x0.2 in particular could be used for further studies to approve its efficacy and safety in the animal model and the patients suffering from inflammatory diseases.

Table 4 The percentage of inhibition by BKE3-NE-T8-x0.2 on LPS induced NO production from RAW264.7 cells and cytotoxicity (Mean ± SEM), (n = 3)

Samples	%Inhibition of Nitric Oxide Production and (%survival)					IC_{50} (µg/ml)
	0.1 µg/ml	1 µg/ml	10 µg/ml	30 µg/ml	50 µg/ml	
NE-base-T8	3.95 ± 2.0 (131.35 ± 15.6)	9.65 ± 3.0 (120.22 ± 12.2)	37.59 ± 4.9 (116.09 ± 16.6)	73.64 ± 3.4 (122.21 ± 7.9)	NT	17.28 ± 2.7
BKE3-NE-T8-x0.2	14.43 ± 3.2 (99.15 ± 8.4)	18.54 ± 2.7 (99.03 ± 15.1)	56.31 ± 5.0 (101.90 ± 15.0)	86.14 ± 1.9 (113.74 ± 20.4)	NT	8.36 ± 1.0*
BKE	NT	-19.83 ± 2.9 (99.48 ± 21.2)	18.52 ± 2.4 (98.21 ± 4.8)	71.42 ± 1.0 (102.11 ± 12.8)	90.69 ± 2.3 (88.34 ± 17.6)	19.72 ± 0.7
Prednisolone	25.19 ± 5.2 (120.94 ± 17.1)	45.20 ± 9.2 (100.98 ± 4.1)	50.36 ± 8.7 (92.31 ± 3.1)	NT	70.63 ± 7.8 (89.81 ± 5.7)	1.72 ± 0.7

*Significant different ($p < 0.05$) compared with BKE ; NT : not tested

Discussions

Benjakul remedy is a traditional Thai medicine containing five herbal plants, i.e., *Piper chaba* Hunt., *Piper sarmentosum* Roxb., *Piper interruptum* Opiz., *Plumbago indica* Linn. and *Zingiber officinale* Roscoe. An ethanolic extract of Benjakul remedy (BKE) prepared in this study showed anti-inflammatory activity via inhibition of NO released from macrophages with the acceptable IC_{50} value of 19.72 ± 0.72 µg/ml. The positive control is prednisolone (IC_{50} value of 1.72 ± 0.7 µg/ml.). This is possible that the anti-inflammatory activity of BKE was mainly from the action of piperine, which is a natural compound found in the pepper plants of Benjakul remedy. Piperine is a potent anti-inflammatory agent. It could inhibit the inflammation processes in the injured tissues by reduction of NO released from macrophages, reduction of PGE2 production and inhibition of IL-6 expression[17, 18]. Therefore, it has been currently used for treatment of inflammation-associated diseases.

Formulations of nanoemulsions containing BKE (BKE-NEs) were developed and characterized their physicochemical properties. The results showed that increase in the Tween 80 content led to the larger droplet size, the higher value of PDI and the less zeta potential. This finding was consistent with the previous study reported by Asasutjarit *et al*.[19] They found that the excess emulsifier molecules could deposit on surface and shielded the charge of the solid lipid nanoparticles. However, these parameters of such nanoparticles were acceptable when using the optimum concentrations of emulsifiers.

Generally, the nanoemulsion formation needs emulsifiers to produce small droplet size of the internal phase by reduction of interfacial tension between oil phase and aqueous phase and protection from droplet aggregation.[20] Lee *et al*.[21] found that the less the emulsifier content, the larger is droplet size of NE. This result might be due to insufficient emulsifier molecules for coating and stabilizing the newly formed droplets and thus coalescence of such droplets occurred. On the other hand, the droplet size of NE was smaller when emulsifier content increased. This finding could be explained that sufficient emulsifier content promoted small droplets of NE with narrow particle size distribution which is desirable.

Xanthan gum was used as a viscosity enhancer. Furthermore, it could control drug release rate from NE bases. In this study, concentrations of xanthan gum were fixed, while the concentration of BKE varied. It was found that the higher concentration of piperine in NE, the faster piperine was released. This result could be explained by the effect of concentration gradients of piperine in the donor- and the receptor compartment of the Franz diffusion cells.

BKE3-NE-T8-x0.2 exhibited stronger anti-inflammation activity than that of BKE and NE-T8-x0.2. These results suggested that NE containing BKE could effectively inhibit NO released from macrophages. Moreover, the synergistic effects of NE base and BKE could lead to the more potent inhibition of inflammatory processes in the injured tissue.

Physicochemical properties of BKE-NE were affected by emulsifier contents. It was found that the optimized BKE-NE containing 10% w/w of mixed oil, 8% w/w of Tween 80 and 0.2% w/w of xanthan gum was first reported with promising physicochemical properties including desirable release profile. Furthermore, these preparations showed high potential for using as topical anti-inflammatory products without skin toxicity. Thus, this BKE-NE could be used for further studies to investigate its efficacy and safety in the animal model.

Acknowledgments

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บทคัดย่อ

ฤทธิ์ต้านการอักเสบของผลิตภัณฑ์นาโนอิมัลชันทาผิวสารสกัดตำรับยาเบญจกูล

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บทนำ: ตำรับยาเบญจกูล เป็นตำรับยาไทยที่อยู่ในบัญชียาหลักแห่งชาติ พ.ศ. 2561 ประกอบด้วยสมุนไพร ใช้เพื่อปรับสมดุลและช่วยบรรเทาอาการท้องอืด การศึกษาก่อนหน้านี้แสดงให้เห็นว่าสารสกัดเบญจกูลชั้นเอทานอลในรูปแบบการรับประทานสามารถช่วยลดอาการปวดในผู้ป่วยโรคข้อเข่าเสื่อม อย่างไรก็ตามสารสกัดตำรับยาเบญจกูลในรูปแบบรับประทานมีผลข้างเคียงต่อระบบทางเดินอาหาร ดังนั้นจึงมีการพัฒนาในรูปแบบนาโนอิมัลชันทาผิวสารสกัดเบญจกูลเพื่อหลีกเลี่ยงผลข้างเคียงที่เกิดขึ้นต่อระบบทางเดินอาหาร

วิธีการศึกษา: สกัดสารตำรับยาเบญจกูลและทดสอบฤทธิ์ต้านการอักเสบโดยการกำหนดฤทธิ์ยับยั้งการปลดปล่อยไนตริกออกไซด์ (NO) ในเซลล์ RAW 264.7 เบญจกูลนาโนอิมัลชันที่มีส่วนประกอบต่างๆ จะถูกเตรียมโดยใช้เทคนิคอัลตราโซนิค และทดสอบสมบัติทางเคมีกายภาพของเบญจกูลนาโนอิมัลชัน ได้แก่ การปลดปล่อยของตัวยา ขนาดของอนุภาค ดัชนีความโปร่งแสง ค่าความต่างศักย์ไฟฟ้า วิทยากระแส สัณฐานวิทยา ทดสอบฤทธิ์ต้านการอักเสบและความเป็นพิษต่อเซลล์ผิว (HaCaT cells)

ผลการศึกษา: สารสกัดชั้นเอทานอลของตำรับยาเบญจกูล มีฤทธิ์ต้านการอักเสบโดยมีค่า IC_{50} เท่ากับ 19.72 ± 0.72 ไมโครกรัม/มิลลิลิตร ในการศึกษาครั้งนี้เบญจกูลนาโนอิมัลชันที่เตรียมมีขนาดอนุภาคประมาณ 25 - 900 นาโนเมตร โดยมีค่าความโปร่งแสงเท่ากับ 0.2 - 1.0 และ ค่าความต่างศักย์ไฟฟ้า -0.8 ถึง -11 mV เบญจกูลนาโนอิมัลชันที่มีส่วนประกอบของสารสกัดเบญจกูล (3%w/w), ทวีน80 (8%w/w) และ แซนแทนกัม (0.2%w/w) แสดงให้เห็นว่าการไหลของผลิตภัณฑ์เป็นแบบ pseudoplastic ลักษณะภายในของเบญจกูลนาโนอิมัลชันมีลักษณะเป็นทรงกลม เบญจกูลนาโนอิมัลชันสามารถยับยั้งการปลดปล่อยไนตริกออกไซด์จากเซลล์ ซึ่งมีค่า IC_{50} เท่ากับ 8.36 ± 1.06 ไมโครกรัมต่อมิลลิลิตร และไม่เป็นพิษต่อเซลล์ผิวหนังในทุกความเข้มข้นที่ทดสอบ

สรุปผลการศึกษา: เบญจกูลนาโนอิมัลชัน มีคุณสมบัติทางเคมีกายภาพที่ดี มีศักยภาพในการต้านการอักเสบเฉพาะที่และไม่มีความเป็นพิษต่อผิวหนัง ดังนั้นจึงสามารถใช้ในการศึกษาต่อในสัตว์ทดลอง

คำสำคัญ: นาโนอิมัลชัน, ตำรับยาเบญจกูล, ฤทธิ์ต้านการอักเสบ, การนำส่งยา, การปลดปล่อยยาเบญจกูล