P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : *https://tci-thaijo.org/index.php/SciTechAsia* Science & Technology Asia
Vol.27 No.1 January - March 2022 Page: [180-189]
Original research article

Anti-inflammatory Activity and Major Compounds of the Traditional Thai Medicines, Triphala, Trikatuk, and their Combined Formulae

Supaluck Nuaeissara[1], Arunporn Itharat[1,2,*], Weerachai Pipatrattanaseree[3], Sumalee Panthong[1]

[1]*Center of Excellence on Applied Thai Traditional Medicine Research, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[2]*Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[3]*Regional Medical Sciences Center 12 Songkhla, Ministry of Public Health, Songkhla 90000, Thailand*

Received 26 March 2020; Received in revised form 29 December 2020
Accepted 15 January 2021; Available online 28 March 2022

ABSTRACT

Inflammation is a common factor leading to pathogenesis of several chronic diseases, including cardiovascular diseases, chronic metabolic syndromes, arthritis, and cancer. Trikatuk and Triphala are traditional poly-herbal preparations widely used in Ayurvedic and Thai traditional medicine. The present study aimed to investigate various combinations of these preparations and their mixing methods to optimize product efficacy. Samples were extracted by boiling in water and maceration with 95% ethanol. All samples were tested for inhibitory effects on *LPS*-induced nitric oxide (NO) production**,** and on *LPS*-induced prostaglandins (PGE_2) from RAW 264.7 cells. Contents of major markers of the extracts were analyzed with HPLC. Trikatuk ethanolic extract showed anti-inflammatory activity through inhibition of NO production but Triphala had no effect on neither NO nor PEG_2 production. A combined formula of Triphala and Trikatuk (1:1) provided moderate anti-inflammatory activity in both pathways. The present study reported the anti-inflammatory effects of various combinations and mixing methods of Trikatuk and Triphala, the results of which could be used to develop optimal combinations for future use as dietary supplements and other health products.

Keywords: Inflammation; Thai traditional medicine; Triphala; Trikatuk

1. Introduction

Inflammation is a biological response system that acts to remove harmful stimuli from the body and to restore normal physiological functions of tissues and organs. Inflammation can be categorized as either

***Corresponding author:** iarunporn@yahoo.com **doi:** 10.14456/scitechasia.2022.18

acute or chronic. Acute inflammation starts rapidly and becomes severe within a short period. However, the body will recover to normal conditions shortly thereafter, unlike chronic inflammation which can eventually cause progressive inflammation, leading to many chronic diseases [1-2]. Inflamed cells play an important role in inflammatory processes, such as in macrophages, leukocytes, mast cells, etc. The inflammatory mediators and cytokines released from these cells, such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and prostaglandins (PG), act as biological defenders neutralizing foreign agents [3]. Inflammation is a common factor leading to pathogenesis of several chronic diseases including cardiovascular diseases, chronic metabolic syndromes, arthritis, and cancer [4]. NO, a free radical released from macrophages, is synthesized by members of the nitric oxide synthase (NOS) enzyme family. Inducible nitric oxide synthases (iNOS) play an important role in producing large amounts of NO during inflammation. Long-term NO exposure of tissue or organs is involved in many diseases such as asthma, rheumatoid arthritis, and atherosclerosis [5-6]. PGs are inflammatory mediators derived from arachidonic acid and metabolized by the cyclooxygenase (COX) pathway [1]. PGs have been detected in many inflammatory diseases including osteoarthritis [7-8]. Prostaglandin E2 (PGE_2) is one of the most abundant PGs produced in the body. PGE_2 is an important mediator of many biological functions, such as immune response regulation, blood pressure modulation, gastrointestinal integrity, and fertility [9].

Traditional medicines derived from medicinal plants have been used for treatments of disease since ancient times. Nowadays, healthcare personnel in modern medicine are increasingly interested in traditional medicines as a new source of treatments for disease. Trikatuk and Triphala are traditional poly-herbal preparations widely used in Ayurvedic and Thai traditional medicine. Trikatuk ('Trikatu' in Ayurveda) is composed of *Piper nigrum* (PN), *Piper chaba* (PC), and *Zingiber officinale* (ZO) at a ratio of 1:1:1. In Ayurvedic medicine, Trikatuk has been used to treat a wide range of illnesses and to increase the bioavailability of other drugs or herbal preparations [10]. Doss and co-workers studied the potential therapeutic effects of Trikatuk in rats with adjuvant-induced arthritis. The results showed that oral administration of Trikatuk suspension (1,000 mg/kg/bw) suppressed the production of pro-inflammatory cytokines (tumor necrosis factor-a (TNF-α), interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein (MCP)-1) and down-regulated the expression of related mRNA [11].

Triphala is composed of *Phyllanthus emblica* (PE), *Terminalia chebula* (TC), and *Terminalia bellirica* (TB) at a ratio of 1:1:1. Researchers have confirmed its anti-inflammatory [12], antimutagenic, radioprotective [13], anticariogenic [14], gastrointestinal health [15], and antineoplastic [16] activities. Recent studies have further demonstrated that it also possesses biological activities such as antiretinopathy [17], anti-cancer cell proliferation [18], skin protection [19], and inhibition of protein fibrillation [20].

Thai Traditional doctors use Trikatuk and Triphala as adaptogenic drugs and prescribe them in different seasons. Trikatuk is prescribed in the rainy season while Triphala is prescribed in the summer. A combination formula of Triphala and Trikatuk has been suggested for use during all seasons. Traditionally, Trikatuk and Triphala are used in a decoction solution form. Nowadays, both Triphala and Trikatuk are sold worldwide as herbal medicines and dietary supplements in various forms such as decoction solution, capsule, and tablet [21-22]. The present study aimed to compare the effects of various preparation methods and to find optimized methods and suitable formulate for anti-inflammatory activity of

Trikatuk and Triphala. It was expected that the results would be useful for further development of traditional medicines or dietary supplements.

2. Materials and Methods

2.1 Chemicals and reagents

Fetal bovine serum (FBS) and trypsin-EDTA were purchased from Gibco® (OK, USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin (P/S), and phosphate buffer saline (PBS) were purchased from Biochrom (MA, Germany). 3-(4, 5-dimethyl -2- thiazolyl-2, 5-diphenyl -2H-tetrazolium bromide (MTT) was purchased from Sigma (MO, USA). Phosphate buffer saline (PBS) was also purchased from Amresco (OH, USA). Dimethyl sulfoxide (DMSO) was purchased from Fluka (Munich, Germany). Prednisolone, piperine, gallic acid, and ellagic acid were purchased from Sigma (MO, USA). Acetonitrile and methanol (HPLC grade) were purchased from RCI LabScan (Bangkok, Thailand). Purified water was prepared by Milli Q® system from Millipore (Bedford, MA, USA). PGE2 Enzyme Immuno-Assay Kit was purchased from Cayman Chemical (MI, USA).

2.2 Plant materials

Piper nigrum L. (fruits; SKP 146 16 14 01), *Piper chaba* Hunt. (fruits; SKP 146 16 03 01), *Zingiber officinale* Roscoe. (rhizomes; SKP 206 26 15 01), *Phyllanthus emblica* Linn. (fruits; SKP 071 16 05 01), *Terminalia chebula* (fruits; SKP 049 20 03 01), *Terminalia bellirica* (fruits; SKP 049 20 02 01) were collected from Kanchanaburi, Thailand. Plant materials were authenticated by comparison with specimens deposited at the herbarium of the Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

2.3 Preparation of crude extracts

Plant materials were cleaned, sliced into small pieces, and dried at 50°C for 24-48 hours. The dried plants were ground into powder with an electric grinder and sieved through a size 60 mesh sieve. The plant powders were stored in air-tight containers at room temperature until use.

Crude extracts of Trikatuk and Triphala were prepared as described in Table 1. The extraction methods used were decoction and maceration with 95% ethanol (3 times x 3 days). The mixing methods for Trikatuk, Triphala, and their combined preparations were as follows. First method, each plant powder was weighed and mixed homogeneously according to the proportion of each formula before extraction. Second method, each plant powder was extracted separately to produce crude extracts which were then weighed and combined according to the formula's proportion (Table 1).

2.4 Inhibitory activity on *LPS*-induced nitic oxide production from RAW 264.7 cells

The inhibition of NO production from RAW 264.7 cells was evaluated following a modified method [23]. Briefly, the cells were seeded in 96-well plates at a density of 1×10^5 cells per well (100 µl) and incubated at 37°C in 5% CO_2 for 24 hrs. The medium was replaced with fresh medium containing 5 ng/ml of LPS (100 µl). Each crude extract was dissolved in DMSO and was diluted to various working concentrations before applying into each well, then the plate was again incubated for 24 hrs. NO production was analyzed by Griess reagent. Aliquots (100 µl) of supernatant in each well were transferred into another 96-well plate to which the same volume of Griess reagent (1% sulfanilamide and 0.1% *N*-(1- naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid) was added. The optical density (OD) was measured by using a microplate reader at a wavelength of 570 nm. The %inhibition of the NO production was

calculated using the following equation, and the IC_{50} values were calculated using a Prism software.

$$\%Inhibition = \left[\frac{OD_{control} - OD_{sample}}{OD_{control}} \right] \times 100,$$

where $OD_{control}$ = mean of control media (+LPS) - mean of control media (-LPS) OD_{sample} = mean of control sample (+LPS) - mean of control sample (-LPS).

2.5 Inhibitory activity on *LPS*-induced prostaglandins (PGE_2) from RAW 264.7 cells

The RAW264.7 cells were seeded into 96-well plates, $1x10^5$ cells/well, and 80 ng/ml of LPS was added to stimulate macrophages, cells were allowed to adhere for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, the supernatant was collected, and the amount of PGE_2 was determined using a PGE_2 enzyme immunoassay kit (Cayman Chemical, MI, USA). The amount of PGE_2 was measured relative to that of the positive control. The %inhibition was calculated using the following equation:

$$\%Inhibition = \left[\frac{OD_{control} - OD_{sample}}{OD_{control}} \right] \times 100.$$

2.6 HPLC analysis of the contents of major markers of the crude extracts

HPLC analysis was done with Agilent 1200 HPLC system (Agilent Technologies, USA) equipped with a solvent degasser (G1322A), a quaternary solvent pump (G1311A), an autosampler (G1329A), a column oven (G1316A), and a photodiode array detector (G1315D). The chromatographic data were processed by Chemstation® software revision B.04.01 SP1.

Chromatographic separation was conducted along a C18 column (4.6 × 150 mm, 5 micron). The mobile phase consisted of acetonitrile (A) and 0.1%v/v phosphoric acid (B) using a linear gradient program as follows: 0-5 min, 5%B; 5 – 40 min, 5%B - 50%B; 40 - 50 min, 50%B - 95%B, 50 -55 min, 95-100%B; 55-60 min, 5%B. The flow rate was set at 1.0 mL/min. Samples of 10 µl each were injected into the HPLC system and detected with diode array detector using a wavelength of 256 nm.

Gallic acid, ellagic acid, and piperine were the major chemical components of Triphala and Trikatuk. All compounds were separated from other peaks showing the specificity of the method. The quantitative analyses of these three compounds were conducted using the constructed calibration curve of standard compounds. The correlation coefficient (r^2) of calibration curves was more than 0.99.

2.7 Data analysis

The results of the anti-inflammatory activity are reported as mean ± standard error of means (SEM), measurements were done in triplicate. IC_{50} values were calculated using regression analysis. Mean differences among groups were analyzed by ANOVA and LSD test. Statistical analysis was conducted using Graphpad Prism software (CA, USA).

3. Results and Discussion

3.1 Inhibitory activities on NO and PGE_2 production from RAW 264.7 cell lines

Results of the inhibitory activity of the ethanolic extracts on the production of NO from RAW264.7 are shown in Fig 1. All sample showed less than 30% cytotoxic effect on RAW 264.7 cells except those of ethanolic extract of TK (TKE and TKEm) and *P. nigrum* (PNE) which showed more than 30% cytotoxicity in the PGE assay at concentrations above 25 µg/ml and 50 µg/ml, respectively. Among the formulae, ethanolic extract of Trikatuk had the greatest efficacy with an IC50 of 19.64±9.31 µg/ml, and 26.53±1.39 µg/ml for TKE and TKEm, respectively. However, all crude extracts

exhibited an inhibitory effect less than the positive control, prednisolone (IC_{50} 0.11±0.02 μg/ml). Among the plant extracts, that of ZOE showed the highest inhibitory activity with an IC_{50} of 17.94±6.32 μg/ml.

Inflammation is a complex process in which immune cells and their soluble factors work together to generate protective responses against infection and cell damage. Further, the inflammatory process involves pro-inflammatory cytokines, chemokines, and inflammatory immune cells [24]. Activation of macrophages is related to the release pro-inflammatory mediators and cytokines, such as NO, PGE_2, interferon-gamma (IFN-γ), TNF-α, IL-1β, and IL-6. Therefore, over-expression of these mediators and cytokines can leads to tissue injury, cell death, and multiple organ failure [25] and are related to many diseases including asthma, cancer, osteoarthritis, rheumatoid arthritis, and diabetes [26].

TKE showed potent inhibitory activity on NO production (19.64±9.30 μg/ml); however, in the assay of the inhibition of PGE_2 production, TKE at concentration >25 μg/ml showed toxicity to the cultured cells. From the results, the combination of Triphala and Trikatuk provide beneficial effects on both anti-inflammatory activities. All combined formulae exhibited moderate inhibitory activity on NO production with IC_{50} values in the range of 30 – 80 μg/ml. The combination of Trikatuk and Triphala provided advantages in terms of efficacy and safety. The anti-inflammatory activity of the combined formula was greater than that of Triphala alone. Although the activity of the combination was less than that of Trikatuk, its toxicity was also less than Trikatuk. As shown in Table 1, all combined formulae did not show cytotoxicity to RAW264.7 cells treated with LPS (80 ng/ml).

Regarding the extracts of the plant components of the formulae, ethanolic extract of ZO (ZOE) showed the strongest inhibitory activity on NO and PGE_2 production with IC_{50} values of 17.94±6.32 μg/ml and 19.53±0.40 μg/ml, respectively. A previous study by Maged and co-workers showed that ethanolic extract of ZO inhibited NO production in RAW 264.7 cells with an IC_{50} of 10 μg/ml [27]. Another study showed inhibitory activity of NO production comparable to our study with an IC_{50} of 20.32±3.23 μg/ml [28]. The traditional Thai Trikatuk medicine uses *Z. mekongense* instead of ZO. However, the present study used ZO because it is more commonly found compared to *Z. mekongense* and it is commonly used in the Thai Trikatuk found in Thai markets. In addition, PN and PC which are plant components of Trikatuk also showed high to moderate inhibitory activity; however, PE, TB, and TC, which are plant components of Triphala, did not show any inhibition.

3.3 Contents of major components of crude extracts

HPLC analysis was a modification of the method used for Trikatuk determination [29]. The gradient mobile phase was optimized to enhance selectivity in the separation of the analytes along the HPLC column. The calibration curves of all compounds showed r^2 greater than 0.99. The retention times of gallic acid, ellagic acid, and piperine were 5, 21, and 42 minutes, respectively. The major compounds of all crude extracts are shown in Table 1. In Triphala, gallic acid and ellagic acid were the two major components, while piperine was the major component in Trikatuk. Therefore, the combined formulae of Triphala and Trikatuk contained these three markers in the chromatogram. The highest content of gallic acid was found in the aqueous extracts of PE (PEW and PEWm), while ellagic acid was the highest in ethanolic extracts of TB (TBE and TBEm). Therefore, in Triphala there were high contents of gallic acid and ellagic acid but not piperine. The highest content of piperine was found in ethanolic extract of PN, which was one of the plant components in Trikatuk, contributing to the high content of

piperine in the ethanolic extract of Trikatuk (TKE and TKEm). This study is the first investigation on combined formulae of Triphala and Trikatuk and on the contents of the major markers and their inhibitory effects on NO and PGE_2 production in RAW264.7 cells.

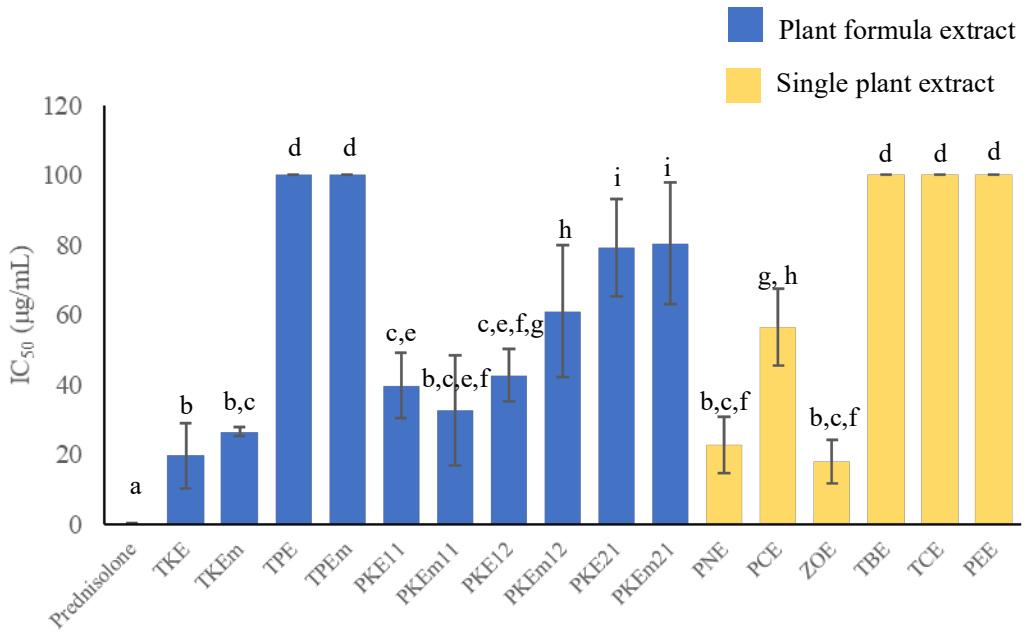

Fig. 1. Inhibition of various crude extracts on NO production in *LPS*-induced RAW 264.7 cells. Mean and SEM values from n=3 are shown. Statistical analysis was performed using ANOVA with LSD test to compare means of each group. Samples labeled with different letters showed significant difference. ($p < 0.05$).

Table1. Major components and IC_{50} of anti-inflammatory effect of the extracts.

Sample	Code Name	Ratio of mixing	Solvent	Code of Sample	Content of compounds mg/g			Inhibitory effects (IC_{50}) (µg/ml)	
					Gallic acid	Ellagic acid	Piperine	Nitric oxide	PGE_2
P. embelica	PE	-	95% Ethanol	PEE	59.74 ±0.53	38.35 ±1.54	-	>100 ^{a}	>100 ^{a}
			Water	PEW	62.11 ±0.39	6.12 ±0.10	-	>100 ^{a}	>100 ^{a}
T. Chebula	TC	-	95% Ethanol	TCE	30.02 ±2.14	12.73 ±1.94	-	>100 ^{a}	>100 ^{a}
			Water	TCW	19.55 ±0.48	-	-	>100 ^{a}	>100 ^{a}
T. bellerica	TB	-	95% Ethanol	TBE	25.82 ±2.37	33.63 ±4.00	-	>100 ^{a}	>100 ^{a}
			Water	TBW	18.54 ±0.60	4.82 ±0.16	-	>100 ^{a}	>100 ^{a}
Z. officinale	ZO	-	95% Ethanol	ZOE	-	-	-	17.94±6.32 ^{b,c,d}	19.53±0.40 ^{b}
			Water	ZOW	-	-	-	>100 ^{a}	>100 ^{a}
P. nigrum	PN	-	95% Ethanol	PNE	-	-	241.62 ±3.06	22.54±8.05 ^{b,c,d}	>50$^{(t)}$

			Water	PNW	-	-	-	>100 [a]	>100 [a]
P.chaba	PC	-	95% Ethanol	PCE	-	-	156.49 ±2.82	56.51±11.00 [e,f]	>100 [a]
			Water	PCW	-	-	-	>100 [a]	>100 [a]
PE:TC:TB (Triphala)		-	1:1:1	95% Ethanol	TPE	39.48 ±1.10	27.15 ±2.37	-	>100 [a]	>50[(t)]
				Water	TPW	30.34 ±1.05	2.36 ±0.35	-	>100 [a]	>100 [a]
ZO:PN:PC (Trikatuk)		-	1:1:1	95% Ethanol	TKE	-	-	165.03 ±4.07	19.64±9.30 [b]	>25[(t)]
				Water	TKW	-	-	-	>100 [a]	>100 [a]
PEE:TCE:TBE		-	1:1:1	95% Ethanol	TPEm	44.59 ±2.61	37.46 ±3.15	-	>100 [a]	>100 [a]
				Water	TPWm	30.79 ±0.46	5.27 ±0.64	-	>100 [a]	>100 [a]
ZOE:PNE:PCE		-	1:1:1	95% Ethanol	TKEm	-	-	143.32 ±4.03	26.53±1.39 [b,c]	>25[(t)]
				Water	TKWm	-	-	-	>100 [a]	>100 [a]
TPE:TKE		-	1:1	95% Ethanol	PKE11	26.18 ±0.97	14.05 ±0.89	81.50 ±3.26	39.62±9.41[c,g]	>100 [a]
TPW:TKW		-		Water	PKW11	17.08 ±0.39	-	-	>100 [a]	>100 [a]
TPEm:TKEm		-	1:1	95% Ethanol	PKEm11	23.95 ±1.27	15.89 ±2.26	76.26 ±1.94	32.60±15.79 [b,c,d,g]	41.39±0.66[b]
TPWm:TKWm		-		Water	PKWm11	16.15 ±0.81	-	-	>100 [a]	>100 [a]
TPE:TKE		-	1:2	95% Ethanol	PKE12	18.31 ±0.28	6.32 ±0.89	95.86 ±0.38	42.57±7.54[c,d,e,g]	>100 [a]
TPW:TKW		-		Water	PKW12	10.42 ±0.14	-	-	>100 [a]	>100 [a]
TPEm:TKEm		-	1:2	95% Ethanol	PKEm12	18.90 ±0.20	5.93 ±0.72	106.37 ±2.53	60.96±18.91 [f]	>100 [a]
TPWm:TKWm		-		Water	PKWm12	11.65 ±0.15	-	-	>100 [a]	>100 [a]
TPE:TKE		-	2:1	95% Ethanol	PKE21	18.31 ±0.28	6.32 ±0.89	95.86 ±0.38	79.35±13.95 [h]	51.00±1.97[c]
TPW:TKW		-		Water	PKW21	10.42 ±0.14	-	-	>100 [a]	>100 [a]
TPEm:TKEm		-	2:1	95% Ethanol	PKEm21	18.90 ±0.20	5.93 ±0.72	106.37 ±2.53	80.40±17.35 [h]	62.22±0.24[d]
TPWm:TKWm		-		Water	PKWm21	11.65 ±0.15	-	-	>100 [a]	>100 [a]
Prednisolone									0.11±0.02 [i]	0.95±0.19[e]

Note: Statistical analysis using ANOVA with LSD test comparing means of each group. Samples labeled with different letters showed significant difference. ($p < 0.05$).
(t) The crude extract showed toxicity to RAW264.7 cells at higher concentrations.

3.4 Comparison of methods of preparation

In the present study, we designed the experimental procedures to determine the preparation methods that would provide the extract with the highest anti-inflammatory activity. We used decoction according to traditional practice [30] and maceration with 95% ethanol [31]. Regarding the mixing methods, in the first method, we combined the plant powders to produce the formula before it was extracted by decoction and maceration. In the second method, each plant powder was extracted by decoction and maceration separately, then the crude extracts of each plant (with the same extraction method) were combined according to the determined formula ratio. As shown in Table 1, all decoction samples showed low anti-inflammatory activity with IC_{50} values of more than 100 μg/ml, while some ethanolic extracts showed moderate to high anti-inflammatory activity. A key limitation of this study was that the formulae combinations were based on the weights of the extracts, not their chemical contents, but the amounts of the chemical components were not equal among the extracts, making it harder to find an optimum combination. Therefore, in future studies, mixing of the extracts based on their extraction yields should be investigated.

4. Conclusion

Combined formulae of Triphala and Trikatuk, traditional Thai medicines used as adaptogens during different seasons, were investigated to obtain a combined formula that could be utilized throughout the year. Trikatuk exhibited inhibitory effects on NO production but showed cytotoxicity when tested for anti-PGE_2 production. Triphala did not show anti-inflammatory activity. However, a combined formula of Triphala and Trikatuk provided advantages superior to the traditional formulae. They exhibited moderate inhibition of NO and PGE_2 production and did not show cytotoxicity when exposed to a high level of LPS. These results provided information for further study of the combined formulae on their protective effects of cells when exposed to hostile environments. The study showed potential anti-inflammatory activity of the combined formulae of Triphala and Trikatuk and provided preparation methods for their future development as traditional medicines or dietary supplements.

Acknowledgements

This project was supported by Thammasat University, the National Research University Project of Thailand Office of Higher Education Commission, Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University, Thailand. The authors gratefully acknowledge Dr. Buncha Ooraikul, Prof. Emeritus, Faculty of Agricultural, Life & Environmental Sciences, University of Alberta and Bualuang ASEAN Chair Professor, Faculty of Medicine, Thammasat University, Thailand for his kind help in grammatical editing and his advice in scientific writing.

References

[1] Ricciotti E, FitzGerald GA. Prostaglandins and Inflammation. Arterioscler Thromb Vasc Biol 2011;31(5): 986-1000.

[2] Choi J. Everything you need to know about inflammation [Internet]. Brighton: Healthline Medial; 2017 [cited 2020 march 10]. Available from: https://www.medicalnewstoday.com/articles/248423

[3] Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S, Syahida A. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J Ethnopharmacol 2004; 92(2-3):263-7.

[4] Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. Nutr Rev 2007;65:S140-6.

[5] Lee J, Yang G, Lee K, Lee MH, Eom JW, Ham I, Choi HY. Antiinflammatory effect of Prunus yedoensis through inhibition of nuclear factork-B in macrophages. BMC Complement Altern M. 2013;13(92):1-9.

[6] Sowndharajan K, Santhanam R, Hong S, Jhoo JW, Kim S. Suppressive effects of acetone extract from the stem bark of three Acacia species on nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. Asian Pac J Trop Biomed 2016; 6(8):658-64.

[7] Sohn DH, Sokolove J, Sharpe O, Erhart JC, Chandra PE, Lahey LJ, et. al. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Tolllike receptor 4. Arthritis Res Ther 2012;14:2-13.

[8] Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, Sokolove J. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016; 12:580-92.

[9] Legler DF, Bruckne M, Allmen E, Krause P. Prostaglandin E2 at new glance: Novel insights in functional diversity offer therapeutic chances. Int J Biochem Cell Biol 2010;42:198-201.

[10] Johri RK, Zutshi U. An Ayurvedic formulation Trikatu and its constituents. J Ethnopharmacol 1992;37(2):85-91.

[11] Doss HM, Ganesan R, Rasool M. Trikatu, an herbal compound ameliorates rheumatoid arthritis by the suppression of inflammatory immune responses in rats with adjuvant-induced arthritis and on cultured fibroblast like synoviocytes via the inhibition of the NFkB signaling pathway. Chem-Biol Interact 2016;258: 175-86.

[12] Kalaiselvan S, Rasool MK. The anti-inflammatory effect of triphala in arthritic-induced rats. Pharm Biol 2015;53(1):51-60.

[13] Takauji Y, Miki K, Mita J, Hossain MN, Yamauchi M, Kioi M, Ayusawa D, Fujii M. Triphala,a formulation of traditional Ayurvedic medicine, shows protective effect against X-radiation in HeLa cells, J biosciences 2016;41(4):569-75.

[14] Bajaj N, Tandon S. The effect of Triphala and Chlorhexidine mouthwash on dental plaque, gingival inflammation, and microbial growth. Int J Ayurveda Res 2011;2(1):29-36.

[15] Rayudu V, Raju AB, Effect of Triphala on dextran sulphate sodium-induced colitis in rats. Ayu 2014;35(3):333-8.

[16] Peterson CT, Denniston K, Chopra D. Therapeutic Uses of Triphala in Ayurvedic Medicine. J Altern Complement Med 2017;23(8):607-14.

[17] Sivasankar S, Lavanya R, Brindha P, Angayarkanni N. Aqueous and alcoholic extracts of Triphala and their active compounds chebulagic acid and chebulinic acid prevented epithelial to mesenchymal transition in retinal pigment epithelial cells, by inhibiting SMAD-3 phosphorylation. PloS One 2015;10(3):1-17.

[18] Cheriyamundath S, Mahaddalkar T, Save SN, Choudhary S, Hosur RV, Lopus M. Aqueous extract of Triphala inhibits cancer cell proliferation through perturbation of microtubule assembly dynamics. Biomed Pharmacother 2018; 98:76-81.

[19] Varma SR, Sivaprakasam TO, Mishra A, Kumar LM, Prakash NS, Prabhu S, Ramakrishnan S. Protective Effects of Triphala on Dermal Fibroblasts and Human Keratinocytes. PloS One 2016;11(1):1-20.

[20] Save SN, Choudhary S. Effects of triphala and guggul aqueous extracts on inhibition of protein fibrillation and dissolution of preformed fibrils. Rsc Adv 2017;7(33): 20460-8.

[21] Kubala J. What are the benefits of Triphala [Internet]. San Francisco : Healthline Media a Red Ventures Company; 2018 [cited 2019 Mar 12]. Available from: https://www.healthline.com/health/triphala-benefits.

[22] Singh J. Trikatu Churna [Internet]. Adelaide: Ayur Times; 2015 [cited 2019 Mar 12]. Available from: https://www.ayurtimes.com/trikatu-churna/

[23] Tewtrakul S, Itharat A. Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. J Ethnopharmacol. 2007;109(3):412-6.

[24] Prabhala HK, Pai C, Prabhala RH. Anti-inflammatory natural foods, in: Watson RR, Preedy VR, Editors. Bioactive food as dietary interventions for arthritis and related inflammatory diseases. 2nd ed. London: Academic Press; 2013. p.279-303.

[25] Pan MH, Chiou YS, Tsai ML, Ho CT. Anti-inflammatory activity of traditional Chinese medicinal herbs. J Tradit Complement Med 2011;1(1):8-24.

[26] Zhang Y, Liu C, Dong B, Ma X, Hou L, Cao X, Wang C. Anti-inflammatory activity and mechanism of surfactin in lipopolysaccharide-activated macrophages. Inflammation 2015;38(2):756-64.

[27] Maged R, Nurul N, Abdulla MS. Anti-inflammatory effects of zingiber officinale roscoe involve suppression of nitric oxide and prostaglandin E2 production. Zanco J Med Sci 2013;17(1):349-56.

[28] 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 (8): S116-24.

[29] Pattanacharoenchai N. Anti-allergic activity of Trikatuk, Triphala and Trisan remedies [dissertation]. Pathumthani: Thammasat University; 2016. 116 p.

[30] Foundation of Restoration and Promotion of Thai Traditional Medicine Ayuravej Vidhyalaya (Jivaka Kormarpaj). Tamra Karn Phat Thai Derm (Phat Sart Songkhroa) 2nd ed. Bangkok: Rongpimp See Thai; 1998. 109p. (in Thai).

[31] Pattanacharoenchai N, Itharat A. Anti-allergic activity of Trikatuk, Triphala and Trisarn remedies. Thammasat Medical Journal 2017;17(4):548-56.