

ANTI-BACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF “YAHOM-NAVAKOT” EXTRACTS, A THAI TRADITIONAL CONCOCTION FOR REDUCING FEVER

Kitrawee Jiraratsatit¹, Arunporn Itharat^{1,2}, Sombat Muengtaweepongsa^{3,4},
Sumalee Panthong¹, Sunita Makchuchit² and Pranporn Kuropakornpong²

¹Department of Applied Thai Traditional Medicine, Faculty of Medicine; ²The Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR); ³Center of Excellence in Stroke, ⁴Department of Internal Medicine, Faculty of Medicine, Thammasat University, Pathum Thani Province, Thailand

Abstract. “Yahom-Navakot” (YN) is a Thai herbal remedy for treating fatigue and post-fever maladies (“Lom Plai Khai”). YN is composed of 55 plant materials based on a Thai essential medicines list. Extracts obtained by maceration with 95% ethanol (YNE) and water decoction (YNW) were analyzed by gas chromatography-mass spectrometry. Anti-bacterial activities of YNE and YNW were based on minimal inhibitory and minimum bactericidal concentrations against a set of four Gram-positive and four Gram-negative bacteria, showing YNE was capable of inhibiting Gram-positive (all but one) but not Gram-negative bacteria while YNW did not exhibit any bactericidal activity. Anti-inflammatory activities of YNE and YNW were examined by the measuring of nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production in lipopolysaccharide-stimulated RAW 264.7 cell line, with YNE exhibiting greater anti-inflammatory potency than YNW. YNE contained greater relative amounts of borneol, β -caryophyllene, 5- α -cholestane, eugenol, fatty acids, and β -selinene compared to YNW, many of which have proven anti-bacterial and anti-inflammatory activities, providing a possible explanation for YNE superior anti-inflammatory property. These findings should assist in furthering studies of bioactive components in YN as possible adjuvant treatments of bacterial infections and inflammation processes.

Keywords: anti-bacterial activity, anti-inflammatory activity, ethanol extract, gas chromatography-mass spectrometry, Thai traditional medicine, water decoction, Yahom-Navakot

Correspondence: Arunporn Itharat, Department of Applied Thai Traditional Medicine, Faculty of Medicine and The Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Thammasat University, Pathum Thani 12120, Thailand
Tel: +66 (0) 2926 9749; Fax: +66 (0) 2926 9749 E-mail: iarunporn@yahoo.com

INTRODUCTION

Infections and inflammations are the main causes of fever (Walter *et al*, 2016), which is treated with antipyretics, *eg* nonsteroidal anti-inflammatory drugs and paracetamol, and antibiotics, *eg* amoxicillin and ciprofloxacin (Doyle and Schortgen, 2016). However, improper use of these drugs can cause adverse side effects, such as heartburn and stomach pain, and drug-resistance can result in prolonged treatment and impact quality of life (Henry, 1988; Granowitz and Brown, 2008). Many studies have reported the efficacy of natural products as an alternative method to treat fever through their anti-bacterial and anti-inflammatory activities (Wu *et al*, 2012; Khan *et al*, 2017). Thai traditional medicine is one of the country's cultural heritages, which has provided alternative effective remedies as evidenced by scientific studies on Thai herbs and concoctions (Chootip *et al*, 2017; Chokpaisarn *et al*, 2019).

"Yahom-Navakot" (YN) is one of the traditional herbal remedies still in current use, being in the Thai essential medicines list under the category treatment of the circulatory system, specifically "Lom Plai Khai"

(NDSDC, 2018). Lom Plai Khai is defined as symptoms, which appear after abatement of fever, such as dizziness, fatigue and body pain. From conventional medicine point of view, Lom Plai Khai could be related to late stages of fever stemming from infection and inflammation. Pyrogens are the main causes of fever, being divided into two major groups, endogenous (cytokines) and exogenous (infections) pyrogens (Dinarello, 2004). Pro-inflammatory cytokines involved fever mechanism include, among others, nitric oxide (NO), prostaglandin E₂ (PGE₂) and tumor necrotic factor-alpha (TNF- α) (Zhang, 2007). NO is capable of inducing inflammatory cytokines and in regulating functional activities of several inflammatory and immune cell types, *viz* macrophages, mast cells, natural killer cells, and neutrophils (Coleman, 2001). In addition, NO can increase body temperature during a fever state (Pritchard *et al*, 2005). PGE₂ is a potent pyrogen, stimulating other pyrogens and cytokines (Coceani *et al*, 1986). TNF- α is a central regulator of inflammation, in particular acute inflammatory processes (Zelova and Hosek, 2013). *In vivo* study in rabbits showed injection of TNF- α induces rise in body temperature during the first

phase of fever (Mabika and Laburn, 1999).

YN ingredients include *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. (Lechner *et al*, 2004), *Picrorrhiza kurroa* Royle ex Benth. (Rathee *et al*, 2016), *Saussurea lappa* (Decne.) Sch. Bip. (Hasson *et al*, 2013), and *Tinospora crispa* (L.) Hook. f. & Thomson (Iqbal *et al*, 2012). Biological activities of YN main ingredient (“Phikud Navakot”) are antioxidative effects (Chiangsom *et al*, 2019; Nalinratana *et al*, 2014), exerting vascular properties (Nusuetrong *et al*, 2012), anti-inflammation effects (in rats) (Gerdprasert *et al*, 2019), protection against ischemia/reperfusion injury (in rats) (Kengkoom *et al*, 2015), anti-lipidemic effects of both YN and Phikud Navakot (Tirawanchai *et al*, 2018).

There are a limited number of investigations on YN chemical components and their respective biological properties (Chootip *et al*, 2017). Here, chemical compositions of YN aqueous and ethanol were investigated using gas chromatography/mass spectrometry (GC/MS) to identify potential anti-bacterial and anti-inflammatory compounds.

MATERIALS AND METHODS

Preparation of YN extracts

YN is composed of 55 plant materials based on the Thai essential medicines list (Table 1). All plant materials, obtained from traditional drugstores in

Bangkok, Thailand, were washed, thinly sliced, and oven-dried at 50°C for 24 hours, then ground and mixed together. Quality of YN powder was assured by methods according to the Thai herbal pharmacopeia guidelines (Department of Medical Sciences, 2017). YN powder (215 g) was macerated with 1000 ml of 95% ethanol for 7 days using a 3000ml macerated bottle (DURAN Group GmbH, Wertheim am Main, Germany), then the extract was filtered through a Whatman filter paper (No. 1) (Merck, Darmstadt, Germany) and filtrate concentrated using a rotary evaporator to yield YNE. Another portion of YN powder (215 g) was boiled in 1000 ml of distilled water for 15 minutes, filtered as described above. The decoction was repeated three time and the combined filtrates were concentrated to 1/3 of its original volume by boiling, then freeze-dried to yield YNW. Both preparations were separately stored at -20°C in an air-tight glass container until used.

GC-MS analysis protocol

GC-MS analysis was performed using a Thermo Focus GC equipped with a polaris Q MS detector, TG-5 slims column and an auto sampler injector (GC-MS; Agilent Technologies, Santa Clara, CA). Injection port temperature was set at 200°C with injection mode split ratio of 50:1 and injection volume of 1.0 µl. Carrier gas was helium. Oven temperature was initially set at 60°C, then increased at a rate of 5°C/minute until 300°C and held at 300°C for 5 minutes. The mass spectrum of each

Table 1
 Plant constituents of “Yahom Navakot”, a Thai traditional herbal remedy for treating post-fever symptoms

Thai name	Common name in English	Scientific name	Part used	Reference for anti-inflammatory activity
Cha-lood	Nut, Lut	<i>Alyxia reinwardtii</i> Bl.	Bark	-
Kra-waan	Camphor seed	<i>Amomum krevanh</i> Pierre ex Gag-nep.	Fruit	Dechayont <i>et al</i> (2019)
Tien-ta-tak-ka-taen	Dill	<i>Anethum graveolens</i> L.	Seed	-
Kote-so	Angelica root	<i>Angelica dahurica</i> Fisch. ex Hoffm.	Root	Pervin <i>et al</i> (2013)
Kote-chiang	Chinese angelica, Tang kuei	<i>Angelica sinensis</i> (Oliv.) Diels	Root	Kim <i>et al</i> (2018)
Krit-sa-na	Agar-wood, Calambac	<i>Aquilaria crassna</i> Pierre	Wood	Wang <i>et al</i> (2018)
Kote-chu-la-lam-pha	Sweet wormwood, Chinese wormwood	<i>Artemisia annua</i> L.	Leaf	Oh <i>et al</i> (2014)
Kote-kha-mao	Atractylis	<i>Atractylodes lancea</i> Thunb.	Rhizome	Jun <i>et al</i> (2018)
Rad-cha-dud	Brucea fruit	<i>Brucea javanica</i> (L.) Merr.	Seed	Yang <i>et al</i> (2013)
Tien-ta-kob	Caraway	<i>Carum carvi</i> L.	Seed	-
Sa-mun-waang	-	<i>Cinnamomum bejolghota</i> (Buch.-Ham.) Sweet	Bark	-
Ob-choei-yuan	Saigon cinnamon	<i>Cinnamomum loureirii</i> Nees	Bark	-

Table 1 (cont)

Thai name	Common name in English	Scientific name	Part used	Reference for anti-inflammatory activity
Phak-chi-ia	Coriander	<i>Coriandrum sativum</i> L.	Fruit	Patel <i>et al</i> (2013)
Tien-khaow	Cumin	<i>Cuminum cyminum</i> L.	Seed	Wei <i>et al</i> (2015)
Haeo-mu	Nut grass	<i>Cyperus rotundus</i> L.	Rhizome	Seo <i>et al</i> (2001); Jung <i>et al</i> (2013)
Sak-khi	Rose wood, Black wood	<i>Dalbergia candenatensis</i> (Dennst.) Prain	Wood	-
Chan-dang	-	<i>Dracaena loureiri</i> Gagnep.	Wood	Itharat <i>et al</i> (2009)
Ya-tin-nok	Itch flower, Bull grass	<i>Eleusine indica</i> (L.) Gaertn.	Tree	-
Ka-lum-pak	Malayan spurge tree	<i>Euphorbia antiquorum</i> L.	Wood	Shih <i>et al</i> (2010)
Tien-khao-plueak	Fennel fruit	<i>Foeniculum vulgare</i> Mill.	Seed	Yang <i>et al</i> (2014); Yang <i>et al</i> (2015)
Cha-em-thet	Sweet root	<i>Glycyrrhiza glabra</i> L.	Root	Li <i>et al</i> (2015); Zhou and Wink (2019)
Kra-dom	-	<i>Gymnopetalum chinensis</i> (Lour.) Merr.	Fruit	-
Ma-li	Jasmine	<i>Jasminum sambac</i> (L.) Aiton	Flower	Widowati <i>et al</i> (2019)
Pror-hom	Lily rhizome	<i>Kaempferia galangal</i> L.	Root	Yao <i>et al</i> (2018)

Table 1 (cont)

Thai name	Common name in English	Scientific name	Part used	Reference for anti-inflammatory activity
Tien-dang	Garden cress seed	<i>Lepidium sativum</i> L.	Seed	Fan <i>et al</i> (2014)
Kote-hua-bua	Szechuan lovage	<i>Ligusticum sinense</i> Oliv.	Root	Itharat <i>et al</i> (2009)
Sa-ra-phi	Negkassar	<i>Mammeas siamensis</i> T. Anders.	Flower	Morikawa <i>et al</i> (2012)
Bun-nak	Indian rose chestnut tree	<i>Mesua ferrea</i> L.	Flower	Teh <i>et al</i> (2017); Murthuza and Manjunatha (2018)
Khon-dok	Bullet wood	<i>Mimusops elengi</i> L.	Wood	Itharat <i>et al</i> (2009)
Phi-kun	Bullet wood	<i>Mimusops elengi</i> L.	Flower	Itharat <i>et al</i> (2009)
Dok-Chan	Mace	<i>Myristica fragrans</i> Houtt.	Mace	-
Look-Chan	Nutmeg	<i>Myristica fragrans</i> Houtt.	Seed	Lee and Park (2011); Dewi <i>et al</i> (2015)
Chan-ted	Sandal wood	<i>Myristica fragrans</i> Houtt.	Wood	-
Kote-cha-da-mang-si	Indian valerain jata-mansi	<i>Nardostachys jatamansi</i> (D. Don) DC.	Flower	Li and Wang (2014); Choi and Jung (2016)
Bua-luang	Lotus stamen	<i>Nelumbo nucifera</i> Gaertn.	Pollen	Moon <i>et al</i> (2019)
Tien-dum	Nigella seed, Fennel flower seed	<i>Nigella sativa</i> L.	Seed	Mahmud <i>et al</i> (2017)

Table 1 (cont)

Thai name	Common name in English	Scientific name	Part used	Reference for anti-inflammatory activity
Ma-kham-pom	Emblic, Malacca, Indian gooseberry	<i>Phyllanthus emblica</i> L.	Fruit	Wang <i>et al</i> (2019)
Kote-kan-praw	Picrorhiza	<i>Picrorhiza kurroa</i> Royle ex Benth.	Rhizome	Kumar <i>et al</i> (2016)
Tien-sat-ta-but	Anise, Anisum	<i>Pimpinella anisum</i> L.	Seed	Conforti <i>et al</i> (2010)
Son	Pine	<i>Pinus kesiya</i> Royle ex Gordon	Wood	Lee <i>et al</i> (2011)
Di-pli	Java long pepper	<i>Piper longum</i> L.	Fruit	Itharat and Panthong (2016); Makchuchit <i>et al</i> (2017)
Sa-khan	-	<i>Piper ribesoides</i> Wall.	Wood	Itharat and Panthong (2016); Makchuchit <i>et al</i> (2017)
Cha-phlu	Betel, Vietnamese pepper	<i>Piper sarmentosum</i> Roxb.	Leaf	Itharat and Panthong (2016); Makchuchit <i>et al</i> (2017)
Tien-kled-hoi	Blond psyllium seed, Indian plantago seed	<i>Plantago ovata</i> Forsk.	Seed	-
Chet-ta-mun-plo-eng-daeng	Rose-colored leadwort, Fire plant	<i>Plumbago indica</i> L.	Root	Itharat and Panthong (2016); Makchuchit <i>et al</i> (2017)

Table 1 (cont)

Thai name	Common name in English	Scientific name	Part used	Reference for anti-inflammatory activity
Pim-sen	Borneol camphor	<i>Pogostemon cablin</i> (Blanco) Benth.	Leaf	Zou <i>et al</i> (2017)
Kote-kra-duk	Kuth root, Costus	<i>Aucklandia lappa</i> DC.	Root	Lim <i>et al</i> (2020)
Sa-ra-phat-phit	Necklace pod	<i>Sophora tomentosa</i> L.	Seed	-
Kan-phlu	Clove, Caryophyllum	<i>Syzygium aromaticum</i> (L.) Merrill & Perry	Flower	Makchuchit <i>et al</i> (2010)
Sa-mor-phi-phek	Beleric myobalan	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Fruit	Jayesh <i>et al</i> (2017)
Kote-phung-pla	Indian nutgall, Chebula gall	<i>Terminalia chebula</i> Retz.	Leaf gall	Jayesh <i>et al</i> (2017)
Bor-ra-petch	Heart leaf moonseed	<i>Tinospora crispa</i> (L.) Hook. f. & Thomson	Vine	Ahmad <i>et al</i> (2018)
Tien-yao-wa-pha-ni	Ajawa seed, Carum	<i>Trachyspermum ammi</i> (L.) Sprague ex Turill	Seed	Shruthi <i>et al</i> (2017)
Phaek-hom	Vetiver, Cuscuta	<i>Vetiveria zizanioides</i> (L.) Nash	Root	Chou <i>et al</i> (2012)
Khing	Ginger	<i>Zingiber officinale</i> Roscoe	Root	Han <i>et al</i> (2013)

peak was recorded and the chemical components were analysed by the Herb and Thai Traditional Medicine Division, BIOTEC Pilot Plant (Thailand Science Park, Bangkok, Thailand). The compounds which were separated by Gas Chromatography were identified by mass values of compounds which were matched with chemical library program.

Anti-inflammatory activity assays

Murine macrophage leukaemia RAW 264.7 cell line (ATCC® TIB-71™) was maintained in Dulbecco's Modified Eagle medium (DMEM) (Difco Laboratories Inc, Franklin Lakes, NJ) supplemented with RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Carlsbad, CA) at 37°C under a humidified atmosphere of 5% CO₂. A 100 µl aliquot cell suspension (1×10⁵ cells) was added to wells of a 96-well plate, incubated for 24 hours as described above, replenished with containing 5 ng/ml lipopolysaccharide (LPS) (Sigma-Aldrich, St Louis, MO) for NO and TNF-α assays or 80 ng/ml LPS (Sigma-Aldrich, St Louis, MO) for PGE2 assay. Then, a 100 µl of YNE or YNW (1, 10, 30, 50, and 100 µg/ml) was added, incubated for 24 hours as described above prior to determination of nitric oxide production. Nitric oxide production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent (2.5% phosphoric acid containing 1% sulfanilamide and 0.1% N(1-naphthyl ethylenediamine

dihydrochloride) (Sigma-Aldrich, St Louis, MO). The absorbance was measured at 540 nm in a microplate reader (Giustarini *et al*, 2008). PGE2 was determined by EIA kit (Cayman Chemical, Ann Arbor, MI) and TNF-α was determined by Mouse TNF-α ELISA test kit (R&D Systems, Minneapolis, MN) respectively. Prednisolone (Sigma-Aldrich, St Louis, MO) was diluted by DMSO (RCI Labscan, Bangkok, Thailand) to be serial dilution (1 mg/ml) and it was used as positive control. Percent inhibition was calculated as follows: $[\text{control value} - \text{sample value}] / \text{control value} \times 100$ and IC₅₀ values were calculated from the Prism program (GraphPad Software, San Diego, CA)

Anti-bacterial activity assay

Test bacteria were selected from common bacterial species known to cause respiratory and gastrointestinal diseases (Kovacs *et al*, 1997): *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pneumococcus aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MRSA DMST 20651, *Staphylococcus epidermidis* ATCC 12228, *Shigella dysenteriae* DMST 15111, and *Salmonella typhi* DMST 22842.

Minimum inhibitory concentration (MIC) assay was modified from a resazurin-in-broth microdilution assay (Sarker *et al*, 2007). Resazurin (Sigma-Aldrich, St Louis, MO) (1 mg/ml 7-Hydroxy-3H-phenoxazin-3-one

10-oxide) (to determine bacterial cell growth) was filtered through a 0.2 μm filter (Sigma-Aldrich, St Louis, MO) and stored at 4°C until used. YNE stock solution prepared in dimethyl sulfoxide and YNW in distilled water were diluted in their respective solvent when used in the assays. A 50 μl aliquot of test sample (0.0001-5 mg/ml) and a 50 μl aliquot of test microbial suspension [1.0×10^5 CFU/ml Mueller Hinton broth (Difco Laboratories Inc, Franklin Lakes, NJ)] were added to wells of a 96-well plate, incubated at 37°C for 18 hours as described above, then a 10 μl aliquot of resazurin solution was added into each well and cell suspension incubated for a further 2-hour period. Bacterial growth reduces resazurin (blue color) to resorufin (pink color) (Chen *et al*, 2015) and minimum concentration of test sample inhibiting pink to blue color change is defined as MIC. Each assay of test sample was performed in triplicate. After determination of MIC, an aliquot of solution from each well was streaked on nutrient agar (Difco Laboratories Inc, Franklin Lakes, NJ), incubated at 37°C for 24 hours and minimum concentration of test sample in well from which no bacterial colony was observed is defined as minimum bactericidal concentration (MBC).

Statistical analysis

Results are reported as mean \pm standard error of the mean (SEM) of three independent experiments. IC_{50} values were calculated using a Prism

program version 5 package (GraphPad Software, San Diego, CA).

RESULTS

GC-MS analysis of Yahom-Navakot extracts

GC-MS analysis of the YN extracts (Fig 1) led to the identification of 12 and 26 chemical compounds in YNW and YNE (Table 2). Predominant constituents of YNE were fatty acids and 5- α -cholestane, while the major aromatic compound was eugenol followed by borneol, and other volatile compounds included β -caryophyllene, hexadecamethyl-cycloheptasiloxane and β -selinene. The predominant constituents of YNW were α -tolunitrile, followed by 7, 9-di-tert-butyl-1-oxaspiro[4.5] deca-6,9-diene-2,8-dione, then 1,11-dihydrododecamethylhexasiloxane, di-tert-butylphenol, tromacaps, and 2,5-diisopropyl-p-xylene. Compounds present in both YNE and YNW were linoleic acid, hexadecamethyl-cycloheptasiloxane and aromatic substances, with a larger amount in the former extract (Table 2).

Anti-inflammatory activities of YN extracts

Anti-inflammatory properties were conducted on LPS-treated murine macrophage leukaemia RAW 264.7 cell line. YNE inhibited production of nitric oxide, PGE2 and TNF- α with IC_{50} value (mean \pm SEM) of 22 ± 1 $\mu\text{g/ml}$, 18 ± 2 $\mu\text{g/ml}$, and 16 ± 3 $\mu\text{g/ml}$, respectively, whereas YNW demonstrated IC_{50} value

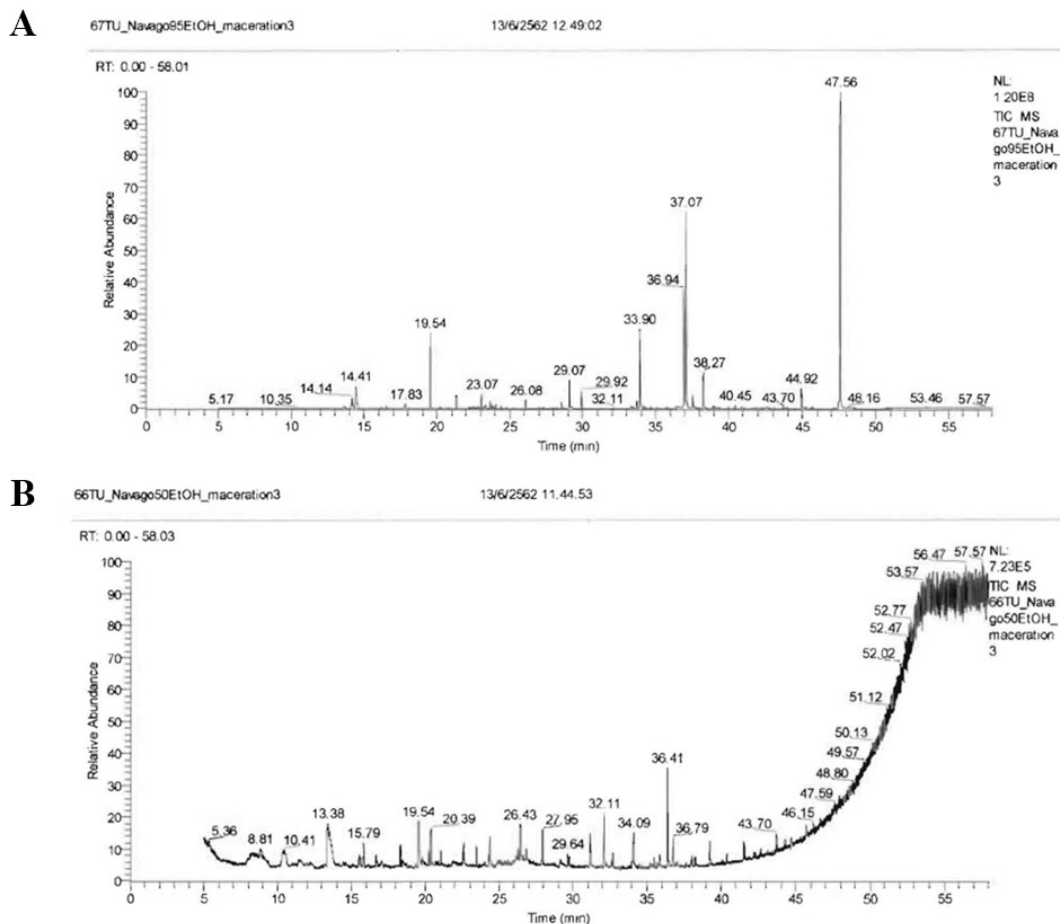


Fig 1 - Gas chromatography-mass spectrometry (GC-MS) profile of “Yahom-Navakot” ethanolic (A) and water (B) extracts

GC-MS analysis was performed using a Thermo Focus GC equipped with a polaris Q MS detector and TG-5 slims column (Agilent Technologies, Santa Clara, CA).

of $IC_{50} > 100 \mu\text{g/ml}$ for all three pro-inflammatory factors and prednisolone control exhibited IC_{50} value of $0.008 \pm 0.612 \mu\text{g/ml}$, $0.073 \pm 0.003 \mu\text{g/ml}$, and $0.073 \pm 0.003 \mu\text{g/ml}$, respectively.

Antibacterial activities of YN extracts

Antibacterial activities of YN extracts were evaluated against four Gram-positive and four Gram-negative bacteria species using both growth

Table 2

Chemical compounds of "Yahom-Navakot" ethanolic (YNE) and water (YNW) extracts identified by gas chromatography-mass spectrometry

Compound	% Chemical compounds	
	YNE	YNW
5- α -Cholestane	32.87	-
Oleic acid	18.99	-
α -Tolunitrile	-	18.76
Linoleic acid	10.81	2.40
Eugenol	7.67	1.51
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	-	7.16
1,11-Dihydrododecamethylhexasiloxane	-	7.08
Di-tert-butylphenol	-	7.08
Palmitic acid	6.36	-
Borneol	3.81	1.47
Tromacaps	-	3.29
Hexadecamethyl cycloheptasiloxane	3.05	1.99
2-Propenoic acid	2.72	-
Myristic acid	1.72	-
β -Selinene	1.42	1.22
β -Caryophyllene	1.33	1.16
Stearic acid	1.29	-
2,5-Diisopropyl-p-xylene	-	1.28
Dillapiole	0.87	-
3-n-Butyl phthalide	0.71	-
Cuminal	0.49	-
Delta-cadinene	0.40	-
n-Docosane	0.38	-

Table 2 (cont)

Compound	% Chemical compounds	
	YNE	YNW
Elemicin	0.34	-
Camphor	0.31	-
Cinnamic acid	0.31	-
Delta-maaliene	0.31	-
1,3,4-Trimethyl-3-cyclohexen-1-carboxaldehyde	0.21	-
Cuparene	0.21	-
α -Muurolene	0.16	-
Ledene	0.13	-
α -Amorphene	0.09	-

in bacterial culture and on nutrient agar. YNE inhibited the Gram-positive bacteria, *S. aureus*, methicillin-resistant *S. aureus* and *S. epidermidis* but not *P. aeruginosa* or any of the Gram-negative bacteria species, whereas YNW did not inhibit any of the eight test bacterial samples (Table 3).

DISCUSSION

YN is a Thai herbal remedy for treating fatigue and fever (NDSDC, 2018). YNE demonstrated higher anti-bacterial and anti-inflammatory activities than YNW. A comparison of chemical constituents using GC-MS allowed us to identify putative compounds in YN extract responsible these properties, *ie* those present in higher amounts or only in one

type of extract compared to the other.

5- α -Cholestane (32.87% in YNE only), the most abundant constituent, has anabolic activity that would be beneficial to patients with Lom Plai Kai (NDSDC, 2018). This followed by organic fatty acids, linoleic acid and oleic acid (10.81 in YNE *vs* 2.40% amount in YNW and 18.99 in YNE *vs* 0% amount in YNW respectively), that reduce production of pro-inflammatory cytokines IL-1 and TNF- α (Almeida *et al*, 2013). A minor organic acid constituent, cinnamic acid (0.31% amount in YNE only), inhibits nitric oxide, PGE2 and TNF- α production in LPS-induced RAW 264.7 cells (Liao *et al*, 2012).

Eugenol (7.67 in YNE *vs* 1.51% amount in YNW) reduces

Table 3
Anti-bacterial activities of “Yahom-Navakot” ethanolic (NYE) and water (YNW) extracts

Sample	<i>Klebsiella pneumoniae</i> ATCC700603	<i>Pneumococcus aeruginosa</i> ATCC 9027	<i>Escherichia coli</i> ECO25922	<i>Staphylococcus aureus</i> ATCC25923	<i>Methicillin-resistant Staphylococcus aureus</i> DMST20651	<i>Staphylococcus epidermidis</i> ATCC 12228	<i>Shigella dysenteriae</i> DMST 15111	<i>Salmonella typhi</i> DMST 22842
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
NYE (mg/ml)	>5.0	-	>5.0	-	2.5	5	1.2	5.0
YNW (mg/ml)	>5.0	-	>5.0	-	>5.0	-	>5.0	-

MBC: minimum bactericidal concentration; MIC: minimum inhibitory concentration

pro-inflammatory cytokines levels of IL-1, IL-6, nitric oxide, PGE2, and TNF- α in vitro (Barboza *et al*, 20118) and effect from murine LPS-induced lung injury (Magalhaes *et al*, 2010). In addition, eugenol has broad-spectrum anti-bacterial activity (Marchese *et al*, 2017) and has been suggested as an adjuvant therapy in treating multi-antibiotic-resistant infection (Marchese *et al*, 2017).

Borneol (3.81 in YNE *vs* 1.47% amount in YNW), a bicyclic monoterpene, is found in many Thai traditional remedy and medicinal plants, such as *Pogostemon cablin* (Blanco) Benth (Feng *et al*, 2019). Borneol reduces inflammation in murine carrageenan-induced peritonitis (Almeida *et al*, 2013) and combined with camphor has bactericidal activity against *Clostridium albicans*, *E. coli* and *S. typhimurium* (Tabanca *et al*, 2001).

Tromacaps (3.29% amount in YNW only), a benzyl isothiocyanate (BITC), inhibits LPS-induced expressions of inducible nitric oxide synthase and COX-2 in RAW 264.7 macrophages (Lee *et al*, 2009), 12-O-tetradecanoyl phorbol-13-acetate-induced murine ear edema (Lee *et al*, 2009). BIT has bactericidal properties against *E. coli* (Nowicki *et al*, 2016), *P. aeruginosa* (Kaiser *et al*, 2017), *S. aureus* (Aires *et al*, 2009), and methicillin-resistant *S. aureus* (Dias *et al*, 2014).

β -Selinene (1.42 in YNE *vs* 1.22% amount in YNW), present in *Callicarpa macrophylla* and

Carum carvi L. (ingredient of YN), inhibits inflammatory processes in carrageenan-induced murine paw edema and has antipyretic effect in mice (Chandra *et al*, 2017). However, there is no previous report on β -selinene on inhibiting specific pro-inflammatory cytokines.

β -Caryophyllene (1.33 in YNE vs 1.16% amount in YNW), a bicyclic sesquiterpene constituent of essential oils, such as clove (*Syzygium aromaticum* (L.) Merrill & Perry) (Batiha *et al*, 2020) and cinnamon (*Cinnamomum loureirii* Nees) (Liang *et al*, 2019), reduces inflammation of carrageenan-induced rat paw edema (Bakir *et al*, 2008) and inhibits production of pro-inflammatory cytokines, such as IL-1 β , IL-6, nitric oxide, and TNF- α , activities of cyclo-oxygenases (COXs)-1 and -2, and NF- κ B activity in activated B cells (Francomano *et al*, 2019). β -Caryophyllene demonstrates potent antibacterial activities against *Bacillus cereus*, *B. subtilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* (Dahham *et al*, 2015).

The present study demonstrates YN has anti-inflammatory property through inhibiting pro-inflammatory cytokines PGE₂, nitric oxide and TNF- α production that are related to both acute and chronic inflammatory processes (Zhang and An, 2007; Landskron *et al*, 2014). This not surprising as the majority of YN plant ingredients have anti-inflammatory activities (Table 1).

The results are in accordance with a previous study on YN main ingredients (Pikad Navakot) demonstrating anti-inflammatory activity (Gerdprasert *et al*, 2019).

In conclusion, the study shows aqueous and ethanolic extracts of "Yahom-Navakot", a Thai traditional herbal remedy for treating post-fever symptoms ("Lom Plai Khai") still in current use, exhibit anti-bacterial and anti-inflammation properties. In addition, identification of constituents present in the two extracts by gas chromatography-mass spectrometry provides data leading to future identification of key bioactive compounds that could be exploited in development of Thai medicinal herbs for adjunct treatment of bacterial infection and inflammatory symptoms.

ACKNOWLEDGEMENTS

The study was supported by a Thailand Integrated Research and Innovation grant and the Center of Excellence in Applied Thai Traditional Medicine Research (CEATMR), Thammasat University. Thammasat University Research Fund and Millimed Co. Ltd. support us with a grant (contract no. TUFT026/2563). Millimed Co. Ltd. and Applied Thai Traditional Center, Faculty of Medicine, Thammasat University supply the study drugs. The author thanks BIOTEC Pilot Plant, Thailand Science Park, for assistance in gas chromatography-mass spectrometry experiments.

REFERENCES

- Ahmad W, Jantan I, Kumolosasi E, Haque MA, Bukhari SNA. Immunomodulatory effects of *Tinospora crispa* extract and its major compounds on the immune functions of RAW 264.7 macrophages. *Int Immunopharmacol* 2018; 60: 141-51.
- Aires A, Mota VR, Saavedra MJ, Rosa EA, Bennett RN. The antimicrobial effects of glucosinolates and their respective enzymatic hydrolysis products on bacteria isolated from the human intestinal tract. *J Appl Microbiol* 2009; 106: 2086-95.
- Almeida JR, Souza GR, Silva JC, et al. Borneol, a bicyclic monoterpene alcohol, reduces nociceptive behavior and inflammatory response in mice. *ScientificWorldJournal* 2013; 2013: 808460.
- Bakir B, Him A, Özbek H, Düz E, Tütüncü M. Investigation of the anti-inflammatory and analgesic activities of β -caryophyllene. *Int J Essent Oil Ther* 2008; 2: 41-4.
- Barboza JN, da Silva Maia Bezerra Filho C, Silva RO, Medeiros JVR, de Sousa DP. An Overview on the anti-inflammatory potential and antioxidant profile of eugenol. *Oxid Med Cell Longev* 2018; 2018: 3957262.
- Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. *Syzygium aromaticum* L. (Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules* 2020; 10: 202.
- Chandra M, Prakash O, Kumar R, et al. β -selinene-rich essential oils from the parts of *Callicarpa macrophylla* and their antioxidant and pharmacological activities. *Medicines (Basel)* 2017; 4: 52.
- Chen JL, Steele TW, Stuckey DC. Modeling and application of a rapid fluorescence-based assay for biotoxicity in anaerobic digestion. *Environ Sci Technol* 2015; 49: 13463-71.
- Chiangsom A, Maniratanachote R, Meksuriyen D, et al. Protective effect of Phikud Navakot extract against hydrogen peroxide-induced oxidative stress in HepG2 cells. *Thai J Pharm Sci* 2019; 43: 186-94.
- Choi S, Jung WS. Anti-inflammatory effects of 8α -hydroxy pinoselinol isolated from *Nardostachys jatamansi* on lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. *Kor J Herbol* 2016; 31: 1-6. [in Korean]
- Chokpaisarn J, Yincharoen K, Sanpinit S, et al. Effects of a traditional Thai polyherbal medicine 'Ya-Samarn-Phlae' as a natural anti-biofilm agent against *Pseudomonas aeruginosa*. *Microb Pathog* 2019; 128: 354-62.
- Chootip K, Chaiyakunapruk N, Soonthornchareonnon N, Scholfield CN, Fuangchan A. Efficacy and safety of "Yahom" as a traditional Thai herbal therapy: a systematic review. *J Ethnopharmacol* 2017; 196: 110-23.
- Chou ST, Lai CP, Lin CC, Shih Y. Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. *Food Chem* 2012; 134: 262-8.

- Coceani F, Bishai I, Lees J, Sirko S. Prostaglandin E2 and fever: a continuing debate. *Yale J Biol Med* 1986; 59: 169-74.
- Coleman JW. Nitric oxide in immunity and inflammation. *Int Immunopharmacol* 2001; 1: 1397-406.
- Conforti F, Tundis R, Marrelli M, *et al.* Protective effect of *Pimpinella anisoides* ethanolic extract and its constituents on oxidative damage and its inhibition of nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J Med Food* 2010; 13: 137-41.
- Dahham SS, Tabana YM, Iqbal MA, *et al.* The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β -caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules* 2015; 20: 11808-29.
- Dechayont B, Phuaklee P, Chunthorng-Orn J, *et al.* Anti-*Helicobacter pylori*, anti-inflammatory and antioxidant evaluation of crude extracts from *Amomum krervanh* fruits. *ScienceAsia* 2019; 45: 109-15.
- Department of Medical Sciences. Thai Herbal Pharmacopoeia 2017. Bangkok: The Agricultural Co-operative Federation of Thailand Ltd; 2017. [in Thai]
- Dewi K, Widyarto B, Erawijantari PP, Widowati W. *In vitro* study of *Myristica fragrans* seed (Nutmeg) ethanolic extract and quercetin compound as anti-inflammatory agent. *Int J Res Med Sci* 2015; 3: 2303-10.
- Dias C, Aires A, Saavedra MJ. Antimicrobial activity of isothiocyanates from cruciferous plants against methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Mol Sci* 2014; 15: 19552-61.
- Dinarelo CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res* 2004; 10: 201-22.
- Doyle JF, Schortgen F. Should we treat pyrexia? And how do we do it? *Crit Care* 2016; 20: 303.
- Fan QL, Zhu YD, Huang WH, Qi Y, Guo BL. Two new acylated flavonol glycosides from the seeds of *Lepidium sativum*. *Molecules* 2014; 19: 11341-9.
- Feng YX, Wang Y, You CX, Guo SS, Du YS, Du SS. Bioactivities of patchoulol and phloroacetophenone from *Pogostemon cablin* essential oil against three insects. *Int J Food Prop* 2019; 22: 1365-74.
- Francomano F, Caruso A, Barbarossa A, *et al.* β -caryophyllene: a sesquiterpene with countless biological properties. *Appl Sci* 2019; 9: 5420.
- Gerdprasert O, Choomchuay N, Chantong B, Sutanthavibul N, Meksuriyen D, Nusuetrong P. Phikud Navakot modulates the level of pro-inflammatory mediators and the protein expression of SOD1 and 2 and the Nrf2/HO-1 signaling pathway in rats with acute myocardial infarction. *Evid Based Complement Alternat Med* 2019; 2019: 4823645.
- Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization. *Methods Enzymol* 2008; 440: 361-80.
- Granowitz EV, Brown RB. Antibiotic adverse

- reactions and drug interactions. *Crit Care Clin* 2008; 24: 421-42.
- Han YA, Song CW, Koh WS, *et al.* Anti-inflammatory effects of the *Zingiber officinale* roscoe constituent 12-dehydrogingerdione in lipopolysaccharide-stimulated Raw 264.7 cells. *Phytother Res* 2013; 27: 1200-5.
- Hasson SS, Al-Balushi MS, Alharthy K, *et al.* Evaluation of anti-resistant activity of *Aucklandia (Saussurea lappa)* root against some human pathogens. *Asian Pac J Trop Biomed* 2013; 3: 557-62.
- Henry DA. Side-effects of non-steroidal anti-inflammatory drugs. *Baillieres Clin Rheumatol* 1988; 2: 425-54.
- Iqbal CM, Gunjan M, Kumar Chellappan D. Antimicrobial activity of *Tinospora crispa* root extracts. *Int J Res Ayurveda Pharm* 2012; 3: 417-9.
- Itharat A, Makchuchit S, Tewtrakul S. Anti-inflammatory activity of Thai traditional medicine preparation called Prasaprophyai. *Planta Med* 2009; 75: 1403-4.
- Itharat A, Panthong S. COX 2 inhibitory activity of Benjakul remedy and its compounds. *Planta Med* 2016; 82 (S 01): S1-S381.
- Jayesh K, Helen LR, Vysakh A, Binil E, Latha MS. Ethyl acetate fraction of *Terminalia bellirica* (Gaertn.) Roxb. fruits inhibits proinflammatory mediators via down regulating nuclear factor-kappaB in LPS stimulated Raw 264.7 cells. *Biomed Pharmacother* 2017; 95: 1654-60.
- Jun X, Fu P, Lei Y, Cheng P. Pharmacological effects of medicinal components of *Atractylodes lancea* (Thunb.) DC. *Chin Med* 2018; 13: 59.
- Jung SH, Kim SJ, Jun BG, *et al.* α -cyperone, isolated from the rhizomes of *Cyperus rotundus*, inhibits LPS-induced COX-2 expression and PGE2 production through the negative regulation of NFkappaB signalling in RAW 264.7 cells. *J Ethnopharmacol* 2013; 147: 208-14.
- Kaiser SJ, Mutters NT, Blessing B, Gunther F. Natural isothiocyanates express antimicrobial activity against developing and mature biofilms of *Pseudomonas aeruginosa*. *Fitoterapia* 2017; 119: 57-63.
- Kengkoom K, Sirimontaporn A, Sotanaphun U, Gerdprasert O, Nusuetrong P. Effects of Phikud Navakot extract on myocardial ischemia/reperfusion injury in rats. *J Med Assoc Thai* 2015; 98 (Suppl 9): S39-47.
- Khan MS, Hamid A, Akram M, *et al.* Antipyretic potential of herbal coded formulation (Pyrexol). *Pak J Pharm Sci* 2017; 30: 195-8.
- Kim YJ, Lee JY, Kim HJ, *et al.* Anti-inflammatory effects of *Angelica sinensis* (Oliv.) Diels water extract on RAW 264.7 induced with lipopolysaccharide. *Nutrients* 2018; 10: 647.
- Kovacs A, Leaf HL, Simberkoff MS. Bacterial infections. *Med Clin North Am* 1997; 81: 319-43.
- Kumar R, Gupta YK, Singh S, Raj A. Anti-inflammatory effect of *Picrorhiza kurroa* in experimental models of inflammation. *Planta Med* 2016; 82:

- 1403-9.
- Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. *J Immunol Res* 2014; 2014: 149185.
- Lechner D, Stavri M, Oluwatuyi M, Pereda-Miranda R, Gibbons S. The anti-staphylococcal activity of *Angelica dahurica* (Bai Zhi). *Phytochemistry* 2004; 65: 331-5.
- Lee JB, Yamagishi C, Hayashi K, Hayashi T. Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from *Pimpinella anisum*. *Biosci Biotechnol Biochem* 2011; 75: 459-65.
- Lee JY, Park W. Anti-inflammatory effect of myristicin on RAW 264.7 macrophages stimulated with polyinosinic-polycytidylic acid. *Molecules* 2011; 16: 7132-42.
- Lee YM, Seon MR, Cho HJ, Kim JS, Park JH. Benzyl isothiocyanate exhibits anti-inflammatory effects in murine macrophages and in mouse skin. *Journal of molecular medicine (Berlin, Germany)* 2009;87(12):1251-61.
- Li C, Eom T, Jeong Y. Glycyrrhiza glabra L. extract inhibits LPS-induced inflammation in RAW macrophages. *J Nutri Sci Vitaminol (Tokyo)* 2015; 61: 375-81.
- Li C, Wang MH. *Nardostachys jatamansi* (D. Don) DC prevents LPS-induced inflammation in RAW 264.7 macrophages by preventing ROS production and down-regulating inflammatory gene expression. *Food Sci Biotechnol* 2014; 23: 903-9.
- Liang Y, Li Y, Sun A, Liu X. Chemical compound identification and antibacterial activity evaluation of cinnamon extracts obtained by subcritical n-butane and ethanol extraction. *Food Sci Nutr* 2019; 7: 2186-93.
- Liao JC, Deng JS, Chiu CS, et al. Anti-inflammatory activities of *Cinnamomum cassia* constituents in vitro and in vivo. *Evid Based Complement Alternat Med* 2012; 2012: 429320.
- Lim JS, Lee SH, Lee SR, et al. Inhibitory effects of *Aucklandia lappa* Decne. extract on inflammatory and oxidative responses in LPS-treated macrophages. *Molecules* 2020; 25: 1336.
- Mabika M, Laburn H. The role of tumour necrosis factor-alpha (TNF-alpha) in fever and the acute phase reaction in rabbits. *Pflugers Arch* 1999; 438: 218-23.
- Magalhaes CB, Riva DR, DePaula LJ, et al. In vivo anti-inflammatory action of eugenol on lipopolysaccharide-induced lung injury. *J Appl Physiol* (1985) 2010; 108: 845-51.
- Mahmud HA, Seo H, Kim S, et al. Thymoquinone (TQ) inhibits the replication of intracellular *Mycobacterium tuberculosis* in macrophages and modulates nitric oxide production. *BMC Complement Altern Med* 2017; 17: 279.
- Makchuchit S, Itharat A, Tewtrakul S. Antioxidant and nitric oxide inhibition activities of Thai medicinal plants. *J Med Assoc Thai* 2010; 93 (Suppl 7): S227-35.

- Makchuchit S, Rattarom R, Itharat A. The anti-allergic and anti-inflammatory effects of Benjakul extract (a Thai traditional medicine), its constituent plants and its some pure constituents using in vitro experiments. *Biomed Pharmacother* 2017; 89: 1018-26.
- Marchese A, Barbieri R, Coppo E, *et al.* Antimicrobial activity of eugenol and essential oils containing eugenol: a mechanistic viewpoint. *Crit Rev Microbiol* 2017; 43: 668-89.
- Moon SW, Ahn CB, Oh Y, Je JY. Lotus (*Nelumbo nucifera*) seed protein isolate exerts anti-inflammatory and antioxidant effects in LPS-stimulated RAW264.7 macrophages via inhibiting NF- κ B and MAPK pathways, and upregulating catalase activity. *Int J Biol Macromol* 2019; 134: 791-7.
- Morikawa T, Sueyoshi M, Chaipech S, *et al.* Suppressive effects of coumarins from *Mammea siamensis* on inducible nitric oxide synthase expression in RAW264.7 cells. *Bioorg Med Chem* 2012; 20: 4968-77.
- Murthuza S, Manjunatha BK. *In vitro* and *in vivo* evaluation of anti-inflammatory potency of *Mesua ferrea*, *Saraca asoca*, *Viscum album* & *Anthocephalus cadamba* in murine macrophages raw 264.7 cell lines and Wistar albino rats. *Beni-Suef Univ J Basic Appl Sci* 2018; 7: 719-23.
- Nalinratana N, Kaewprem W, Tongumpai S, Luechapudiporn R, Sotanaphun U, Meksuriyen D. Synergistic antioxidant action of Phikud Navakot ameliorates hydrogen peroxide-induced stress in human endothelial cells. *Integr Med Res* 2014; 3: 74-82.
- National Drug System Development Committee (NDSDC). Thailand National List of Essential Medicines (NLEM) 2018, [cited 2020 Jan 19]. Available from: URL: http://ndi.fda.moph.go.th/uploads/main_drug_file/20180529192247.pdf [in Thai]
- Nowicki D, Rodzik O, Herman-Antosiewicz A, Szalewska-Palasz A. Isothiocyanates as effective agents against enterohemorrhagic *Escherichia coli*: insight to the mode of action. *Sci Rep* 2016; 6: 22263.
- Nusuetrong P, Sotanaphun U, Tep-Areenan P. Effects of Phikud Navakot extract on vascular reactivity in the isolated rat aorta. *J Med Assoc Thailand* 2012; 95 (Suppl 12): S1-7.
- Oh YC, Jeong YH, Kim T, Cho WK, Ma JY. Anti-inflammatory effect of *Artemisiae annuae herba* in lipopolysaccharide-stimulated RAW 264.7 cells. *Pharmacog Mag* 2014;10 (Suppl 3): S588-95.
- Patel D, Desai S, Gajaria T, Devkar R, Ramachandran AV. *Coriandrum sativum* L. seed extract mitigates lipotoxicity in RAW 264.7 cells and prevents atherogenic changes in rats. *EXCLI J* 2013; 12: 313-34.
- Pervin M, Hasnat MA, Debnath T, Park SA, Kim DH, Lim BO. Antioxidant, anti-inflammatory and antiproliferative activity of *Angelica dahurica* root extracts. *J Food Biochem* 2013; 38: 281-92.
- Pritchard MT, Li Z, Repasky EA. Nitric oxide production is regulated by fever-range thermal stimulation of

- murine macrophages. *J Leukoc Biol* 2005; 78: 630-8.
- Rathee D, Rathee P, Rathee S, Rathee D. Phytochemical screening and antimicrobial activity of *Picrorrhiza kurroa*, an Indian traditional plant used to treat chronic diarrhea. *Arab J Chem* 2016; 9 (Suppl 2): S1307-13.
- Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* 2007; 42: 321-4.
- Seo WG, Pae HO, Oh GS, *et al.* Inhibitory effects of methanol extract of *Cyperus rotundus* rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. *J Ethnopharmacol* 2001; 76: 59-64.
- Shih MF, Cheng YD, Shen CR, Cherng JY. A molecular pharmacology study into the anti-inflammatory actions of *Euphorbia hirta* L. on the LPS-induced RAW 264.7 cells through selective iNOS protein inhibition. *J Nat Med* 2010; 64: 330-5.
- Shruthi RR, Venkatesh YP, Muralikrishna G. Structural and functional characterization of a novel immunomodulatory glycoprotein isolated from ajowan (*Trachyspermum ammi* L.). *Glycoconj J* 2017; 34: 499-514.
- Tabanca N, Kirimer N, Demirci B, Demirci F, Baser KH. Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phrygia* and the enantiomeric distribution of borneol. *J Agric Food Chem* 2001; 49: 4300-3.
- Teh SS, Ee GC, Mah SH. Evaluation of nitric oxide inhibition effect in LPS-stimulated RAW 264.7 macrophages by phytochemical constituents from *Mesua beccariana*, *Mesua congestiflora*, and *Mesua ferrea*. *Med Chem Res* 2017; 26: 3240-6.
- Tirawanchai N, Supapornhemin S, Somkasetrin A, Suktitipat B, Ampawong S. Regulatory effect of Phikud Navakot extract on HMG-CoA reductase and LDL-R: potential and alternate agents for lowering blood cholesterol. *BMC Complement Altern Med* 2018; 18: 258.
- Walter EJ, Hanna-Jumma S, Carraretto M, Forni L. The pathophysiological basis and consequences of fever. *Crit Care* 2016; 20: 200.
- Wang HM, Fu L, Cheng CC, *et al.* Inhibition of LPS-induced oxidative damages and potential anti-inflammatory effects of *Phyllanthus emblica* extract via down-regulating NF- κ B, COX-2, and iNOS in RAW 264.7 cells. *Antioxidants (Basel)* 2019; 8: 270.
- Wang S, Yu Z, Wang C, Wu C, Guo P, Wei J. Chemical constituents and pharmacological activity of agarwood and *Aquilaria* plants. *Molecules* 2018; 23: 342.
- Wei J, Zhang X, Bi Y, Miao R, Zhang Z, Su H. Anti-inflammatory effects of cumin essential oil by blocking JNK, ERK, and NF- κ B signaling pathways in LPS-stimulated RAW 264.7 cells. *Evid Based Complement Alternat Med* 2015; 2015: 474509.

- Widowati W, Prahastuti S, Ekayanti N, *et al.* Anti-inflammation assay of black soybean extract and its compounds on lipopolysaccharide-induced RAW 264.7 cell. *J Phys Conf Ser* 2019; 1374: 012052.
- Wu ZW, Wang ZM, Lu Y, *et al.* Comparative study on anti-inflammatory and antipyretic effects between Dao-di herb and non Dao-di herb of Huangqin. *Zhongguo Zhong Yao Za Zhi* 2012; 37: 3628-32. [in Chinese]
- Yang IJ, Lee DU, Shin HM. Anti-inflammatory and antioxidant effects of coumarins isolated from *Foeniculum vulgare* in lipopolysaccharide-stimulated macrophages and 12-O-tetradecanoylphorbol-13-acetate-stimulated mice. *Immunopharmacol Immunotoxicol* 2015; 37: 308-17.
- Yang IJ, Yu HY, Lee DU, Shin HM. Anti-inflammatory effects of the fruits of *Foeniculum vulgare* in lipopolysaccharide-stimulated macrophages. *J Life Sci* 2014; 24: 981-7.
- Yang J, Li S, Xie C, *et al.* Anti-inflammatory activity of ethyl acetate fraction of the seeds of *Brucea javanica*. *J Ethnopharmacol* 2013; 147: 442-6.
- Yao F, Huang Y, Wang Y, He X. Anti-inflammatory diarylheptanoids and phenolics from the rhizomes of kencur (*Kaempferia galanga* L.). *Ind Crop Prod* 2018; 125: 454-61.
- Zelova H, Hosek J. TNF- α signalling and inflammation: interactions between old acquaintances. *Inflam Res* 2013; 62: 641-51.
- Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin* 2007; 45: 27-37.
- Zhou JX, Wink M. Evidence for anti-inflammatory activity of isoliquiritigenin, 18 β glycyrrhetic acid, ursolic acid, and the traditional chinese medicine plants *Glycyrrhiza glabra* and *Eriobotrya japonica*, at the molecular level. *Medicines (Basel)* 2019; 6: 55.
- Zou L, Zhang Y, Li W, *et al.* Comparison of chemical profiles, anti-inflammatory activity, and UPLC-Q-TOF/MS-based metabolomics in endotoxic fever rats between synthetic borneol and natural borneol. *Molecules* 2017; 22: 1446.