Original Articles

Anti-inflammatory and antioxidant activities of the Thai traditional remedy called "Leard-ngam" and its plant ingredients

Chakrapanpong Threrapanithan, Nuanjan Jaiaree, Arunporn Itharat, Sunita Makchuchit, Pakakrong Thongdeeying, Sumalee Panthong

Abstract

Introduction: Thai traditional medicine has used Leard-ngam remedy (LG) for the treatment of primary dysmenorrhea including pain and inflammation. LG is a dysmenorrhea preparation on the Thai National List of Herbal Medicinal Products A.D. 2011. Objective of the present study was to investigate anti-inflammatory and anti-oxidant activities of LG remedy, and its herbal components.

Method: LG remedy was extracted similarly to that practiced by Thai traditional doctors (ethanol and water extraction). These extracts were tested for their inhibition of nitric oxide (NO) production and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

Result: Three ethanolic extracts of *Piper nigrum* Linn., *Zingiber officinale* Roscoe., *Citrus hystrix* DC. and 95% ethanolic extract of LG showed potent anti-inflammatory activity, with IC_{50} values of 1.31 ± 0.42, 2.87 ± 0.31, 3.03 ± 3.27 and 28.18 ± 4.63 µg/ml, respectively, where IC_{50} of the positive control, Indomethacin was 25.04 ± 3.79 µg/ml. The DPPH radical scavenging assay showed that ethanolic extracts of *Syzygium aromaticum* Linn.Merr. Et Perry, *Oroxylum indicum* (Linn.) Kurz., *Zingiber officinale* Roscoe. exhibited strong antioxidant activity, with EC_{50} values of 9.20 ± 0.29, 9.94 ± 0.91 and 14.34 ± 0.28 µg/ml, respectively, whereas the standard butylated hydroxytoluene (BHT) showed mean EC_{50} value of 15.84 ± 1.42 µg/ml.

Discussion and Conclusion: It is concluded that LG remedy and its herbal components show high inhibitory effect against NO production with strong radical scavenging activity. These results support the use of LG and herb components in Thai traditional medicine.

Key words: Anti-inflammatory, Free radical scavenging activity, Leard-ngam remedy

Received: 14 May 2015 Accepted: 3 July 2015

Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University

Corresponding Author: Chakrapanpong Threrapanithan, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand e-mail: champ_kub@hotmail.com

Introduction

Dysmenorrhea, one of the most frequently encountered gynecologic disorders, refers to painful menstruation. It typically occurs in the first few years after menarche[1] and affects as many as 50% of post pubertal females. In Thailand dysmenorrhea is the most prevalent complaint in women (42.2%)[2]. Dysmenorrhea is classified as primary or secondary dysmenorrhea[3, 4]. Primary dysmenorrhea is defined as menstrual pain that is not associated with macroscopic pelvic pathology (i.e, occurs in the absence of pelvic disease). Secondary dysmenorrhea is defined as menstrual pain resulting from anatomic or macroscopic pelvic pathology, as is seen in women with endometriosis or chronic pelvic inflammatory disease[3, 4]. Leard-ngam remedy (LG) has been used for treatment of primary dysmenorrhea including pain and inflammation. LG is a dysmenorrhea preparation in the Thai National List of Herbal Medicinal Products A.D. 2011. LG is used by Thai traditional practitioners in hospitals and medical clinics[5]. In addition to LG, Thai traditional practitioners also used the other herbs such as, *Piper nigrum, Piper sarmentosum, Piper retrofractum, Zingiber officinale, Zingiber cassumunar* to treat primary dysmenorrhea including pain. The previous research showed that each ethanolic extracts of *P. nigrum, P. retrofractum* and *P. indica* exhibited inhibitory effect on nitric oxide production in RAW 264.7[6]. Thus, this study was to investigate the anti-inflammatory and antioxidant activities of Leard-ngam remedy, its ingredients and herb components. The results support the use of Thai traditional medicine to reduce pain and inflammation in primary dysmenorrhea.

Method

Animal cell lines and Reagent

RAW 264.7 murine macrophage leukemia cell lines were established and kindly provided by Assoc. Prof. Dr.Supinya Tewtrakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. RPMI Medium 1640 (RPMI1640) Powder with L glutamine, Fetal Bovine Serum (FBS), Penicillin-Streptomycin (P/S), Tripsin-EDTA and trypan blue were purchased from Gibco, USA. Phosphate Buffer Saline (PBS) was from Amresco (USA), sodium bicarbonate was from BDH (England), lipopolysaccharide (LPS, from *Escherichia coli*), 3-(4,5-dimethy-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) were from Sigma (USA).

Preparation of the plant extracts

LG remedy consisted of *Allium sativum* Linn., *Amomum xanthioides* Wall., *Artemisia vulgaris* L., *Boesenbergia rotunda* Linn., *Citrus aurantifolia* (Christm.et Panz. Swingle)., *Citrus hystrix* DC., *Cymbopogon citratus* (DC.Stapf)., *Glycyrrhiza glabra* L. *Metha cordifolia* Opiz., *Myristica fragrans* Houtt., *Ocimum sanctum* Linn., *Oroxylum indicum* (L.) Kurz., *Piper nigrum* L., *Piper retrofractum* Vahl., *Piper sarmentosum* Roxb., *Plumbago indica* L., *Syzygium aromaticum* (Linn.) Merrill and Perry., *Zingiber cassumunar* Roxb., *Zingiber officinale* Roscoe. and *Zingiber zerumbet* (L.) Smith. and the ratio of which were mixed as shown in Table 1. LG preparation and its plant ingredients were extracted in a similar way to those practiced by Thai traditional doctors, There were divided into two equal parts; the first part was macerated in 95% ethanol for 3 days, 3 times and percentage of yield, while the second part was boiled in distilled water at boiling point for 15 minutes, 3 times. All of extracts concentrated to dryness under reduced pressure. The crude extracts were then pooled and kept in freezer (-20°C) until required. All extracts were studied for anti-inflammatory and free radical scavenging activities.

Table 1 The ethnobotanical data

Scientific Name	Common name	Family name	Part used	Ratio in remedy (Percentages)
Allium sativum Linn.	Garlic	ALLIACEAE	Bulb	5
Amomum xanthioides Wall.	Bustard cardamom	ZINGIBERACEAE	Seed	5
Artemisia vulgaris Linn.	Mugwort /Common wormwood	ASTERACEAE (COMPOSITAE)	All parts	5
Boesenbergia rotunda (Linn.) Mansf.	Fingerroot	ZINGIBERACEAE	Rhizome	5
Citrus aurantifolia Christm. et Panz.Swingle	Common lime	RUTACEAE	Leaf	5
Citrus hystrix DC.	Leech lime	RUTACEAE	Peel	5
Cymbopogon citratus (DC.) Stapf	Lemongrass	POACEAE (GRAMINEAE)	All parts	5
Glycyrrhiza glabra Linn.	Licorice	FABACEAE (LEGUMINOSAE)	Root	5
Mentha cordifolia Opiz.	Kitchen mint	LAMIACEAE (LABIATAE)	All parts	5
Myristica fragrans Houtt.	Nutmeg tree	MYRISTICACEAE	Seed	5
Ocimum sanctum Linn.	Holy basil	LAMIACEAE (LABIATAE)	Leaf	5
Oroxylum indicum (Linn.) Kurz	Broken bones tree	BIGNONIACEAE	Bark	5
Piper nigrum Linn.	Pepper	PIPERACEAE	Seed	5
Piper retrofractum Vahl.	Long pepper	PIPERACEAE	Flower	5
Piper sarmentosum Roxb.	Wildbetal leafbush	PIPERACEAE	All parts	5
Plumbago indica Linn.	Rose- colored Leadwort	PLUMBAGINACEAE	Root	5
Syzygium aromaticum Linn. Merrill and Perry	Clove	MYRTACEAE	Flower	5
Zingiber cassumunar Roxb.	Cassumunar ginger	ZINGIBERACEAE	Rhizome	5
Zingiber officinale Roscoe	Ginger	ZINGIBERACEAE	Rhizome	5
Zingiber zerumbet (Linn.) Smith.	Shampoo ginger	ZINGIBERACEAE	Rhizome	5

Assay for inhibitory effect on nitric oxide production[7]

Inhibitory effect on Nitric oxide (NO) production in mouse leukaemic macrophage cell line (RAW 264.7) was studied. Briefly, the RAW 264.7 cell line was cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. Cells were harvested using trypsin-EDTA and diluted to a suspension in fresh medium, and seeded into 96-well plates to obtain 1 x 10^5 cells/well and allowed to adhere for 1 h at 37°C in a humidified atmosphere containing 5% CO_2. After that, the medium was replaced with fresh medium containing test sample at various concentrations and then incubated for 48 hours. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. The supernatant (100 µl) was removed and added to 96-well plates. Griess reagent (100 µl) was add to 96-well plates with a microplate reader at 570 nm. The stock solution of each test sample was dissolved in DMSO, and the solution was added into the medium RPMI (final DMSO is 2%). To calculate the inhibition (%) by using the following equation IC_{50} values were determined by Prism program (n = 3):

Inhibition (%) = ((A-C)-B / A-C) x 100

[A: 2 %DMSO +LPS, C: 2 %DMSO +Media, B: Sample –blank]

IC_{50} value (effective concentration of sample required to inhibit NO release for 50%) was obtained by linear regression analysis of dose-response cure plotting between % inhibition and concentrations and calculated by Prism program. The 95% ethanolic extracts of LG remedy, its fractions and herb components were tested for anti-inflammatory effects, starting with concentrations of 1, 10, 50 to 100 µg/ml. The extract showing more than 50% inhibition of NO production was evaluated for their anti-inflammatory activities and toxicity compared to the positive controls (Indomethacin and MTT assay).

Cytotoxicity activity by MTT assay[7, 8]

Mouse leukaemic macrophage cell line (RAW 264.7). Briefly, the RAW 264.7 cell line was cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin. Cells were harvested using trypsin-EDTA and diluted to a suspension in fresh medium. Cells were then seeded in 96-well plates to obtain 1 x 10^5 cells/well and allowed to adhere for 1 hour at 37°C in a humidified atmosphere containing 5% CO_2. After that the medium was replaced with fresh RPMI medium containing test sample at various concentrations and then incubated for 48 hours. The supernatant was add to 96-well plates. Cytotoxicity was determined using the 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. Briefly, after 48 hours incubation with test samples, MTT solution (10 µl, 5 mg/ml in PBS) was added to each wells. After 2 hours incubation, the medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan production in the cells. Optical density of formazan solution was measured with a microplate reader at 570 nm, where test compound was considered cytotoxic when the optical density of the formazan solution was less than 80% of that of the control (vehicle-treated) group. Indomethacin was used as positive control. The stock solution of each test sample was dissolved in DMSO, and the solution was added to the medium RPMI (final DMSO is 2%). Percent inhibition was calculated using the following equation, and IC_{50} values was determined by Prism program. The experiment was done as triplicate. The result was expressed as the percentage of growth inhibition (% Cytotoxicity) and IC_{50} by mean and standard error of mean (SEM).

$$\% \text{ Cytotoxicity} = [(OD_{control} - OD_{sample})/OD_{control}] \times 100$$

[OD = Optical density]

DPPH radical scavenging method[9]

DPPH (1, 1-dipheny1-2-picrylhydrazyl) is considered as an unstable radical because of the paramagnetism conferred by its odd electron. The solution (in absolute ethanol) has a deep violet color and shows a strong absorption band at 520 nm. The DPPH radical can accept an electron or hydrogen radical to become a stable diamagnetic molecule, in which the absorption vanishes and the resulting decolorization is stoichiometric with the number of electrons taken up (Blois, 1958). A DPPH solution having concentration of $6x10^{-5}$ M was used in the present study since at this low concentration the color is not too dense and the Lambert-Beer law is obeyed. Samples for testing were dissolved in absolute ethanol to obtain final concentrations of 1, 10, 50 and 100 µg/ml of the extract. Each concentration was tested in triplicate. A portion of sample solution (500 µg) was mixed with an equal volume of $6x10^{-5}$ M DPPH (in absolute ethanol) and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 520 nm. BHT (butylated hydroxytoluene), a well-known synthetic antioxidant was tested in the same system as a positive standard. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The experiment was done in triplicate and expressed as the EC_{50} by mean and standard error of mean (SEM). The result was expressed as percentage inhibition.

$$\% \text{ Inhibition} = [(OD_{control} - OD_{sample})/OD_{control}] \times 100$$

[OD = Optical density]

Bioassay guide fractionation

The 95% ethanolic extract of LG remedy show anti-inflammatory activity. Bioassay guided fractionation was performed extract with good biological activities was studied. An aliquot of the ethanolic extract of Leard-ngam remedy (50 g) was separated by vacuum liquid chromatography (VLC), using hexane (2,000 ml) (LG_a), hexane : chloroform (1 : 1, 2,000 ml) (LG_b), chloroform (2,000 ml) (LG_c), chloroform : methanol (1 : 1, 2,000 ml) (LG_d) and methanol (1,500 ml) (LG_e) and drying by rotary evaporation. All ethanolic fractions of LG remedy were studied for anti-inflammatory and free radical scavenging activities.

Result

The percentage yields, anti-inflammatory and anti-oxidant activities of LG remedy and its fractions and herb components were shown in Table 2.

Table 2 Anti-inflammatory and antioxidant activities of LG remedy and its herb components

Plant name	Code*	Percentage yield (%)	Free radical scavenging activity (EC_{50} ± SEM)[a]	Anti-inflammatory activity (IC_{50} ± SEM)[a]
Allium sativum Linn.	AS1	0.77	> 100	48.78 ± 4.65
	AS2	62.24	> 100	> 100
Amomum xanthioides Wall.	AX1	1.00	37.2 ± 2.94	97.82 ± 4.23[b]
	AX2	7.50	11.91 ± 0.83	> 100
Artemisia vulgaris Linn.	AV1	5.62	95.60 ± 2.28	8.52 ± 3.42[b]
	AV2	21.97	41.61 ± 1.27	> 100
Boesenbergia rotunda (Linn.) Mansf.	BR1	2.70	> 100	4.92 ± 1.43[b]
	BR2	3.22	> 100	43.85 ± 1.29
Citrus aurantifolia	CA1	5.00	> 100	> 100
	CA2	4.34	> 100	> 100
Citrus hystrix DC.	CH1	17.60	> 100	3.03 ± 3.27[b]
	CH2	20.41	> 100	> 100
Cymbopogon citratus (DC.) Stapf	CC1	2.63	> 100	85.05 ± 3.74[b]
	CC2	5.04	61.20 ± 2.57	> 100
Glycyrrhiza glabra Linn.	GG1	6.95	26.20 ± 0.93	5.83 ± 1.32[b]
	GG2	25.17	78.64 ± 2.62	7.15 ± 3.28[b]
Mentha cordifolia Opiz.	MC1	4.35	16.98 ± 0.67	8.46 ± 3.28[b]
	MC2	7.79	18.49 ± 0.99	> 100
Myristica fragrans Houtt.	MF1	12.16	19.29 ± 2.83	48.10 ± 3.45[b]
	MF2	3.56	50.05 ± 3.13	> 100
Ocimum sanctum Linn.	OS1	5.77	37.39 ± 2.10	42.10 ± 3.18[b]
	OS2	6.73	16.68 ± 1.67	> 100

Table 2 Anti-inflammatory and antioxidant activities of LG remedy and its herb components (continue)

Plant name	Code*	Percentage yield (%)	Free radical scavenging activity (EC_{50} ± SEM)[a]	Anti-inflammatory activity (IC_{50} ± SEM)[a]
Oroxylum indicum (Linn.) Kurz	OI1	2.77	9.94 ± 0.91	5.64 ± 0.45
	OI2	15.25	32.26 ± 1.30	> 100
Piper nigrum Linn.	PN1	4.46	55.54 ± 2.84	1.31 ± 0.42[b]
	PN2	6.20	> 100	> 100
Piper retrofractum Vahl.	PR1	8.30	> 100	3.48 ± 0.23[b]
	PR2	7.88	> 100	> 100
Piper sarmentosum Roxb.	PS1	2.80	64.72 ± 4.57	10.96 ± 0.65
	PS2	10.30	> 100	> 100
Plumbago indica Linn.	PI1	6.54	24.58 ± 1.12	7.54 ± 2.41[b]
	PI2	13.70	69.06 ± 4.13	69.49 ± 3.51[b]
Syzygium aromaticum Linn.Merr. Et Perry	SA1	14.15	9.20 ± 0.29	51.86 ± 3.61[b]
	SA2	20.22	5.83 ± 0.17	21.00 ± 0.57
Zingiber cassumunar Roxb.	ZC1	10.75	58.03 ± 4.90	4.93 ± 0.42
	ZC2	9.32	> 100	> 100
Zingiber officinale Roscoe	ZO1	4.69	14.34 ± 0.28	2.87 ± 0.31
	ZO2	7.82	93.50 ± 3.50	73.78 ± 2.31
Zingiber zerumbet (Linn.) Smith.	ZZ1	4.75	> 100	22.18 ± 2.89[b]
	ZZ2	9.85	> 100	41.13 ± 2.46
Leard-ngam remedy	LG1	8.67	48.80 ± 3.95	28.18 ± 4.63
	LG2	17.15	53.24 ± 5.28	> 100
	LG3	4.50	56.57 ± 5.20	> 100
Leard-ngam remedy	LG_a	0.68	> 100	> 100
(VLC Fraction)	LG_b	1.13	> 100	> 100
	LG_c	23.23	61.26 ± 2.07	> 100
	LG_d	59.96	32.74 ± 1.95	23.94 ± 0.71
	LG_e	7.77	46.59 ± 3.43	> 100
Butylated hydroxytoluene	BHT	-	15.84 ± 1.42	-
(Standard control)		-		
Indomethacin	IDM	-	-	25.04 ± 3.79

[a] Values were mean ± S.E.M. (n = 3).

[b] Cytotoxic effect was observed by MTT assay (% Survival < 70 at concentration 100 μg/ml)

*Code number 1 was 95% ethanolic extract, 2 was water extract and 3 was 50% ethanolic extract.

As shown in Table 2, determination of inhibitory activity on nitric oxide production showed that ethanolic extract of LG remedy exhibited NO production inhibitory activity, where most water extracts were apparently inactive (IC_{50} > 100 µg/ml.) except for *Syzygium aromaticum* Linn. Mer. Et Perry which showed potent inhibitory activity, with IC_{50} value of 21.00 ± 0.57 µg/ml. Ten ethanolic extracts namely of *Piper nigrum* Linn., *Zingiber officinale* Roscoe, *Citrus hystrix* DC., *Piper retrofractum* Vahl., *Boesenbergia rotunda* (Linn.) Mansf., *Zingiber cassumunar* Roxb., *Oroxylum indicum* (Linn.) Kurz, *Glycyrrhiza glabra* Linn., *Plumbago indica* Linn. and *Piper sarmentosum* Roxb. showed potent inhibitory activity. Interestingly, these plants extracts exhibited NO production inhibitory effect higher than the positive control, Indomethacin (IC_{50} value of 25.04 ± 3.79 µg/ml). The 95% ethanolic extract of LG showed NO inhibitory effect with IC_{50} value of 28.18 ± 4.63 µg/ml. LG_d chloroform : methanol (1:1) fraction showed inhibitory effect with IC_{50} value of 23.94 ± 0.71 µg/ml and high percentage of yield (59.96%).

For DPPH radical scavenging activity, showed that three ethanolic extracts namely *Syzygium aromaticum* Linn. Merr. Et Perry., *Oroxylum indicum* (Linn.) Kurz and *Zingiber officinale* Roscoe. showed higher antioxidant activity than the standard BHT, whereas the 95% ethanolic extract of LG preparation had low antioxidant activity with a mean EC_{50} value of 48.80 ± 3.95 µg/ml.

Discussion and Conclusion

This investigation of LG remedy is based on the use by Thai traditional doctors to treat inflammation which is the leading cause of dysmenorrhea. This study showed that the 95% ethanolic extracts of LG and herb components (*Zingiber officinale* Roscoe., *Zingiber cassumunar* Roxb., *Oroxylum indicum* (Linn.) Kurz., *Piper sarmentosum* Roxb., and *Syzygium aromaticum* Linn.Merr. Et Perry.) exhibited high inhibition on NO production compared with standard (Indomethacin). These results support the use of Thai traditional medicine for treating inflammation which is the leading cause of dysmenorrhea in the medical health care system thus can be explained scientifically.

Acknowledgements

This project was supported by funding from Thammasat University. The authors gratefully acknowledge the financial and logistic supports from Department of Applied Thai Traditional Medicinal and Herbal Medicine & Food Unit, Faculty of Medicine, Thammasat University, Thailand.

References

1. Lemeke DP, Pattison J, Marshall LA, Cowley DS. Primary care of women. Norwalk Conn. Appleton & Lange: 1992;420-32.
2. Dawood MY. Nonsteroidal anti-inflammatory drugs and changing attitudes toward dysmenorrhea. Am J Med 1988;84:23-9.
3. Jamieson DJ, Steege JF. The association of sexual abuse with pelvic pain complaints in a primary care population. Am J Obstet Gynecol 1997;177:1408-12.
4. Dawood MY. Dysmenorrhea. J Reprod Med 1985;30:154-67.
5. The Institute of Thai Traditional Medicine. List of Herbal Medicinal Products A.D. 2011. Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health of Thailand 2011; 1:56.
6. Kakatum N, Jaiarree N, Makchucit S, Itharat A. Anti-oxidant and anti-inflammatory activities of Thai medicinal plants in Sahasthara remedy for muscle pain treatment. J Med Assoc Thai 2012;95:120-6.
7. Tewtrakul S, Subhadhirasakul S, Karalai C, Ponglimanont C, Cheenpracha S. Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*. Food Chemistry 2009;115:534-8.
8. Tewtrakul S, Itharat A. Anti-allergic substances from the rhizomes of *Dioscorea membranacea*. Bioorganic & Medicinal Chemistry 2006;14:8707-11.
9. Yamazaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the anti-oxidative effect of methanol extracts of crude drugs. Chem Pharm Bull 1994;42:1663-5.

บทคัดย่อ

ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระของตำรับยาเลือดงาม และส่วนประกอบในตำรับ

จักรพรรณพงษ์ ธีรปณิธาน, นวลจันทร์ ใจอารีย์, อรุณพร อิฐรัตน์, ศุณิตา มากชูชิต, ผกากรอง ทองดียิ่ง, สุมาลี ปานทอง

สถานการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

ผู้ให้ติดต่อ นายจักรพรรณพงษ์ ธีรปณิธาน สถานการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

อีเมล champ_kub@hotmail.com

บทนำ: การแพทย์แผนไทยตั้งแต่อดีตจนถึงปัจจุบันมีการใช้ตำรับยาเลือดงามเพื่อรักษาอาการปวดท้องประจำเดือน ลดการอักเสบมาช้านาน และต่อมาได้ถูกบรรจุอยู่ในบัญชียาหลักแห่งชาติ เมื่อปี พ.ศ. ๒๕๕๔ วัตถุประสงค์เพื่อศึกษา ฤทธิ์ต้านการอักเสบ ฤทธิ์ต้านอนุมูลอิสระ ของสารสกัดจากตำรับยาเลือดงามสมุนไพรเดี่ยวในตำรับ และสารที่แยกได้จากตำรับเลือดงาม

วิธีการศึกษา: ตำรับยาเลือดงาม และส่วนประกอบในตำรับจะสกัดโดยวิธีเดียวกับการใช้ของแพทย์แผนไทย คือ การหมักด้วย 95% เอทานอลและการต้มน้ำ หลังจากนั้นจะนำสารสกัดทั้งหมดมาทดสอบฤทธิ์ต้านการอักเสบโดยการยับยั้งการหลั่งไนตริกออกไซด์ และฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging assay

ผลการศึกษา: ผลการทดสอบฤทธิ์ต้านการอักเสบพบว่าสารสกัดชั้นเอทานอลของพริกไทย ขิง มะกรูด และตำรับยาเลือดงามมีฤทธิ์ต้านการอักเสบโดยมีค่า IC_{50} เท่ากับ ๑.๓๑ ± ๐.๔๒, ๒.๘๗ ± ๐.๓๑, ๓.๐๓ ± ๓.๒๗ และ ๒๘.๑๘ ± ๔.๖๓ ไมโครกรัมต่อมิลลิลิตร ตามลำดับ เมื่อเทียบกับสารมาตรฐานคือ Indomethacin ซึ่งมีค่า IC_{50} เท่ากับ ๒๕.๐๔ ± ๓.๗๙ ไมโครกรัมต่อมิลลิลิตร สำหรับฤทธิ์ต้านอนุมูลอิสระพบว่าสารสกัดชั้นเอทานอลของกานพลู เพกา และขิง ซึ่งเป็นส่วนประกอบในตำรับมีฤทธิ์ดีกว่าสารมาตรฐาน Butylated hydroxytoluene (BHT) โดยมีค่า EC_{50} เท่ากับ ๙.๒๐ ± ๐.๒๙, ๙.๙๔ ± ๐.๙๑, ๑๔.๓๔ ± ๐.๒๘ และ ๑๕.๘๔ ± ๑.๔๒ ไมโครกรัมต่อมิลลิลิตร ตามลำดับ

วิจารณ์ และสรุปผลการศึกษา: ผลการศึกษานี้แสดงให้เห็นว่าตำรับยาเลือดงาม และสมุนไพรที่เป็นส่วนประกอบในตำรับมีฤทธิ์ต้านการอักเสบและต้านอนุมูลอิสระได้ จึงเป็นข้อมูลสนับสนุนการใช้ยาไทยและสมุนไพรได้ต่อไป

คำสำคัญ: ฤทธิ์ต้านการอักเสบ, ฤทธิ์ต้านอนุมูลอิสระ, ตำรับยาเลือดงาม