

Original Article

Phytochemical analysis of Leard–Ngam remedy and its composition

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Abstract

Introduction: Leard–Ngam remedy (LG) has long been used for treating primary dysmenorrhea and balancing women's health. It consists of twenty herbs. The quality test and phytochemical analysis are important for authentication and identification of the herbal products.

Objective: To analyze the phytochemical components of LG remedy and quantify the components

Method: Phytochemical components of LG remedy were analyzed by using gas chromatography-mass spectrometry (GC-MS) in order to identify and quantify the phytochemical components. Loss on drying, total ash, extractive value, acid-insoluble ash and toxic heavy metals determinations were used for quality assessment of LG remedy and its plant ingredients. Heavy metal, Arsenic (As), Lead (Pb) and Cadmium (Cd) were determined by atomic absorption spectrometry.

Result: The study found that eugenol, austrobailignan, acetueugenol and piperine were found as phytochemical compounds of LG remedy. This remedy passed the standard guidelines of loss on drying, total ash, acid insoluble ash and heavy metals. However, some medicinal plants of LG remedy fell below standard; firstly, *A. sativum* and *A. vulgaris* did not meet the total ash content, may be caused by calcium oxalate. For drug preparation, *B. rotunda* using from rhizome and *O. sanctum* from root, the contamination of gravels and rocks from soil were judged to be caused by falling in standardization. LG remedy and twenty herbs passed the standard guidelines of heavy metal.

Conclusion: Phytochemical compound of LG remedy were eugenol, austrobailignan, acetueugenol and piperine. Some single herbs which consisted of LG remedy were less than some of the standardization. However, when combined as a remedy, it passed all standards.

Key word: Leard–Ngam remedy, standardization, phytochemical analysis

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Introduction

Leard-Ngam remedy (LG) is an herbal remedy for women's health. LG remedy is used for curing primary dysmenorrhea, leukorrhea and balancing women's health. LG remedy is listed on the National List of Essential Medicines, 2018 and consists of twenty herbs. They are *Allium sativum* Linn, *Amomum xanthioides* Wall, *Artemisia vulgaris* Linn, *Boesenbergia rotunda* (Linn.) Mansf, *Citrus aurantifolia* Christm et Panz.Swingle, *Citrus hystrix* DC, *Cymbopogon citratus* (DC.) Stapf, *Glycyrrhiza glabra* Linn, *Mentha cordifolia* Opiz, *Myristica fragrans* Houtt, *Ocimum sanctum* Linn, *Oroxylum indicum* (Linn.) Kurz, *Piper nigrum* Linn, *Piper retrofractum* Vahl, *Piper sarmentosum* Roxb, *Plumbago indica* Linn, *Syzygium aromaticum* Linn.Merr et Perry, *Zingiber cassumunar* Roxb, *Zingiber officinale* Roscoe and *Zingiber zerumbet* (Linn.) Smith.¹ Past research reported the biological activities of many herbs composing LG remedy, for example *Z. officinale* has [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol that can inhibit PGE₂ production in RAW 264.7 macrophage cells with IC₅₀ value of 6 µM². The hexane extract of *Z. cassumunar* showed inhibition on carrageenan-induced rat paw edema as well as on the exudate formation, leukocyte accumulation and prostaglandin biosynthesis in carrageenan-induced rat pleurisy³, *B. rotunda* exhibited the inhibition of NO production⁴. The methanol extract of *Z. zerumbet* inhibited PGE₂ induced rat ileum at concentrations of 5 and 10 mg/mL⁵. For safety and efficacy of herbal remedy used in clinical trial, the quality standards and chemical

analysis are important, the standard tests are moisture content, total ash, acid insoluble ash and extractive value⁶. Although, details of many herbs were reported in Thai Herbal Pharmacopoeia 2019, there has been no report for quality control and phytochemical analysis of LG remedy. Therefore, this study aimed to determine the phytochemical constituents of LG remedy by using gas chromatography-mass spectrometry (GC-MS) in order to identify and quantify, followed by a quality assessment on LG remedy and its plant ingredient for standardizing and preparing LG remedy to continue in further clinical trials.

Methods

Plant Materials

Preparation of single herbs and sample solution

Twenty herbs of LG remedy were bought from reliable sources as shown in Table 1. LG remedy consists of twenty herbal plants, namely *A. sativum*, *A. xanthioides*, *A. vulgaris*, *B. rotunda*, *C. aurantifolia*, *C. hystrix*, *C. citratus*, *G. glabra*, *M. cordifolia*, *M. fragrans*, *O. sanctum*, *O. indicum*, *P. nigrum*, *P. retrofractum*, *P. sarmentosum*, *P. indica*, *S. aromaticum*, *Z. cassumunar*, *Z. officinale*, and *Z. zerumbet*. All plants were cleaned, sliced and dried at 50 °C in an oven and ground to powder. The dried powder of each plant was macerated with 95% ethanol. The LG remedy extract was concentrated to dryness under reduced pressure. The crude extracts (LG remedy) were then pooled and kept in a freezer (-20°C) until use for chemical analysis.

Table 1 Thai medicinal herbs in Leard-Ngam remedy

NO.	Scientific name	Family	Thai's name	Part of use	Source	Specimen NO.
1	<i>Allium sativum</i> L.	LILIACEAE	Krathiam	Bulb	Chiang-mai province	SKP 006 01 19 01
2	<i>Amomum xanthioides</i> Wall	ZINGIBERACEAE	Reaw	Seed	China	SKP 206 01 24 01
3	<i>Artemisia vulgaris</i> L.	COMPOSITAE	Kot Chulalumpa	All parts	China	SKP 051 01 01 01
4	<i>Boesenbergia rotunda</i> (L.) Mansf	ZINGIBERACEAE	Krachai	Root	Suphan Buri Province	SKP 095 02 18 01
5	<i>Citrus aurantifolia</i> (Christm.) Swingle	RUTACEAE	Ma Naow	Leaf	Ratchaburi Province	SKP 166 03 01 01
6	<i>Citrus hystrix</i> DC.	RUTACEAE	Makrut	Peel	Ratchaburi Province	SKP 166 03 08 01
7	<i>Cymbopogon citratus</i> (DC.)	GRAMINEAE	Tra Krai Ban	All parts	Pathum Thani Province	SKP 081 03 03 01
8	<i>Glycyrrhiza glabra</i> L.	LEGUMINOSAE	Cha Aim Tes	Root	Kanchanaburi Province	SKP 072 07 07 01
9	<i>Mentha cordifolia</i> Opiz.	LAMIACEAE	Sa Ra Nae	All parts	Kanchanaburi Province	SKP 095 13 03 01
10	<i>Myristica fragrans</i> Houtt.	MYRISTICACEAE	Luk-Jan	Seed	China	SKP 121 13 06 01
11	<i>Ocimum sanctum</i> Linn.	LABIATAE	Kra Prow	Leaf	Suphan Buri Province	SKP 095 15 19 01
12	<i>Oroxylum indicum</i> Linn	BIGNONIACEAE	Pae Ka	Bark	Kanchanaburi Province	SKP 025 15 09 01
13	<i>Piper nigrum</i> Linn.	PIPERACEAE	Phrik Thai Lon	Seed	Chanthaburi Province	SKP 146 16 14 01
14	<i>Piper retrofractum</i> Vahl	PIPERACEAE	Di Pli	Flower	Kanchanaburi Province	SKP 146 16 18 01
15	<i>Piper sarmentosum</i> Roxb.ex Hunter	PIPERACEAE	Chaphlu	All parts	Kanchanaburi Province	SKP 146 16 19 01
16	<i>Plumbago indica</i>	PLUMBAGINACEAE	Chettamool Pleang Dang	Root	Kanchanaburi Province	SKP 148 16 09 01
17	<i>Syzygium aromaticum</i> (L.)	MYRTACEAE	Kan Phlu	Flower	Indonesia	SKP 123 19 01 01
18	<i>Zingiber cassumunar</i> Roxb.	ZINGIBERACEAE	Phlai	Rhizome	Kanchanaburi Province	SKP 206 26 03 01
19	<i>Zingiber officinale</i> Roscoe	ZINGIBERACEAE	Khing	Root	Phetchabun Province	SKP 206 26 15 01
20	<i>Zingiber zerumbet</i> (L.) Sm.	ZINGIBERACEAE	Kra Teaw	Rhizome	Kanchanaburi Province	SKP 206 26 26 01

2.2 Phytochemical components of the ethanolic extract of Leard-ngam remedy by using gas chromatography-mass spectrometry (GC-MS)

The ethanol extract of LG remedy (LG) was analyzed by using a Thermo focus GC Gas Chromatography-Mass Spectrometry with capillary column TG-5 Slims (30 m x 0.25 mm x 0.25 µm), (Thermo Fisher Scientific). The ionization energy was achieved by electron impact at 70 eV. Helium (He) gas was the carrier with flow rate 1.0 ml/min. The initial temperature of column oven was programmed for 60 °C, and then heated to 300 °C with a rate of 5 °C/min and kept constant at 300 °C for 10 min. The mass spectrum of each peak was recorded in the positive ion current mode of mass spectrometer within a mass range of 35 to 400. Identification of oil constituents was achieved using the Central Scientific Instrument Center (CSIC) TU Science.

2.3 Determination of Quality control

Standards for quality control as defined in Thai Herbal Pharmacopoeia contribute to high quality of herbal drug as follows; loss on drying (moisture content), total ash and acid insoluble ash, extractive value and toxic heavy metals (Arsenic (As), Cadmium (Cd), Lead (Pb)).

2.3.1 Loss on drying (moisture content)⁶

An electronic moisture analyzer was used to determine the loss on drying. Five grams of the dried powder of each plant were placed in the moisture analyzer for 30 minutes then analyzing and recording the data.

2.3.2 Total ash⁶

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non-physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. A crucible had been prepared by

cleaning and placing in hot air oven at 105°C for 5 hours until weight of crucible was stable. About 2 grams of the ground dried material was placed in a crucible and a burned crucible by using muffle furnace at 450°C for 9 hours until the powder changed to grey color of ash. The crucible was then placed in a desiccator to cool down. Then it was returned to the muffle furnace at 450°C for 5 hours and again cooled in desiccator. This was repeated until the crucible weight was stable. Total ash was calculated using the following formula:

$$\% \text{ Total ash} = \frac{\text{Stable weight after burning (g)}}{\text{Weight before burning (g)}} \times 100$$

The standard value of Thai herbal pharmacopoeia indicates that total ash content not more than 10 %w/w or depend on each herbs.

2.3.3 Acid insoluble ash⁶

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. In this procedure; total ash was put in beaker, 10% HCl was added and boiling for 5 minutes, filtered using Whatman paper No. 42 and the paper washed with distilled water until pH was 7. The filter paper was placed in a crucible and burned in muffle furnace at 500°C for 9 hours until the weight of crucible was stable. Acid insoluble ash was calculated in percentage using the following formula:

$$\% \text{ Acid insoluble ash} = \frac{\text{Stable weight after burning (g)}}{\text{Weight before burning (g)}} \times 100$$

The standard value of Thai herbal pharmacopeia indicates that acid insoluble ash is < 2% w/w after burning at 550°C.

2.3.4 Heavy metals⁶

The final form of plant material were recommended that the heavy metal; lead (Pb), arsenic (As) and cadmium (Cd) levels are not more than 10, 4 and 0.3 ppm, respectively measured by atomic absorption spectrometry

2.3.5 Extractive value

2.3.5.1 Ethanol soluble extractive value⁷

Five grams of the dried powder of each of the plants were macerated in 100 ml of ethanol, in closed flask and specific strength, for 24 hours, shaking frequently during the first 6 hours and then allowing it to stand for 18 hours. The extract was filtered rapidly to avoid losing ethanol and dried 20 ml of the filtrated extract in a tarred, flat-bottomed, shallow dish at 105°C to give constant weight.

$$\% \text{ Extractive value} = \frac{\text{Weight of the extract (g)}}{\text{Weight of dried powder of plant (g)}} \times 100 \times 5$$

2.3.5.2 Water soluble extractive value⁷

The water soluble extract was performed using chloroform water as same as described in ethanol soluble extractive value.

Result

3.1 Chemical analysis of LG remedy

The chemical analysis by using gas chromatography-mass spectrometry (GC-MS) as shown in Table 2 and Figure 1 found twenty-five known chemical compounds and forty-three unknown compounds from ethanol extract of LG remedy. The major phytochemical compounds were eugenol, austrobailignan, acetueugenol, and piperine with content of 31.22%, 9.44%, 6.57%, and 5.89 %, respectively.

Table 2 Chemical compounds detected from ethanolic extract of Leard-ngam remedy

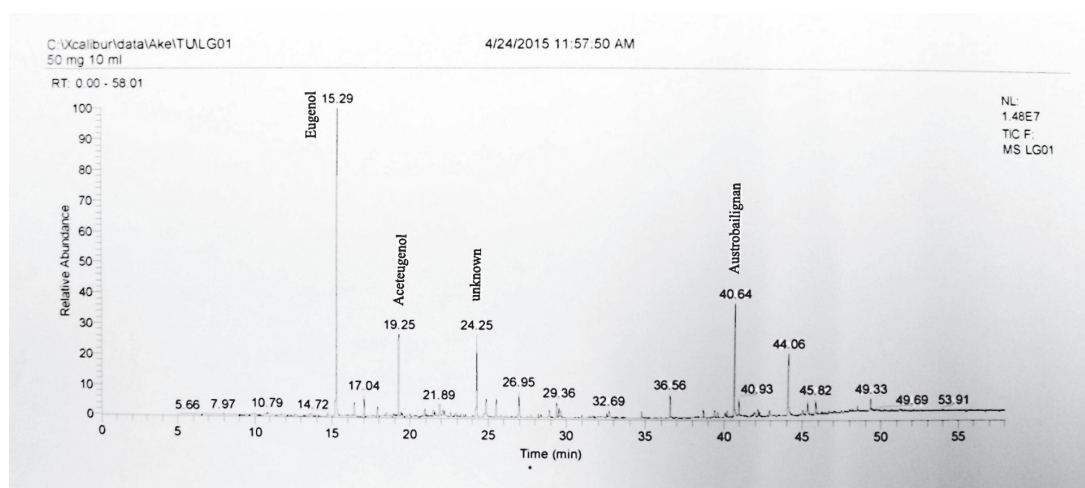
LG remedy		
RT	Active compounds	%Area
9.93	Camphor	0.22
10.79	Terpinene-4-ol	0.43
11.68	Coumaran	0.12
12.59	Chavicol	0.20
13.51	Iso-bornyl acetate	0.15
13.63	Beta-isosafrole	0.29
14.72	Phenylpropionic acid	0.30
15.29	Eugenol	31.22
15.92	Copaene	0.14
16.43	Methyl Eugenol	1.16
17.04	Beta-caryophyllene	1.94
17.92	Alpha-Guaiene	0.94
19.07	2,4-Di-tert-butylphenol	0.15
19.25	Aceteugenol	6.57
20.00	Elemicin	0.33
20.95	Caryophyllene oxide	0.73

Table 2 Chemical compounds detected from ethanolic extract of Leard-ngam remedy (Continue)

LG remedy		
RT	Active compounds	%Area
21.04	Phenol,2-(1-methyl-2-butenyl)-4-methoxy	0.32
21.51	cis-Asarone	0.50
21.89	3,4-Dimethoxyphenylbutadiene (DMPBD)	1.13
22.10	Unknown	0.68
22.19	Unknown	0.47
22.60	Unknown	0.44
23.26	Unknown	0.30
23.56	Unknown	0.22
24.04	Unknown	0.20
24.25	Unknown	6.86
24.89	Myristic acid	1.71
25.52	Unknown	1.41
26.95	(2E)-3-(2,3-Dimethoxyphenyl)-2-propenoic acid	2.19
27.74	Unknown	1.41
28.19	Unknown	0.35
28.37	Unknown	0.36
28.90	Palmitric acid	0.75
29.36	3,4-Dimethoxyphenylbutadiene (DMPBD)	1.30
29.52	Unknown	0.70
29.62	Unknown	0.56
30.98	Unknown	0.24
31.35	Unknown	0.08
31.49	Unknown	0.20
31.76	Unknown	0.02
31.99	Unknown	0.14
32.18	Unknown	0.19
32.56	Unknown	0.32
32.69	Unknown	0.63
34.76	Unknown	0.59
36.56	Pinostrobin chalcone	2.18
38.35	Unknown	0.19
38.68	Unknown	0.81
39.39	Unknown	0.86
39.59	Unknown	0.47
40.08	Unknown	0.40
40.18	Unknown	0.61
40.64	Austrobailignan	9.44

Table 2 Chemical compounds detected from ethanolic extract of Leard-ngam remedy (Continue)

LG remedy		
RT	Active compounds	%Area
40.93	Unknown	1.70
41.69	Unknown	0.26
41.82	Unknown	0.44
41.93	Unknown	0.66
42.16	Unknown	0.69
42.26	Unknown	0.42
42.91	Unknown	0.59
44.06	Piperine	5.89
45.01	Unknown	0.61
45.29	Unknown	1.48
45.82	Unknown	1.39
46.21	Unknown	0.28
46.67	Unknown	0.35
47.47	Unknown	0.34
48.49	Unknown	0.62
49.33	Unknown	1.32

**Figure 1** LG remedy, s chromatogram analysis by GC-MS

3.2 The Quality control

Quality parameters including total ash, acid insoluble ash, loss on drying, extractive value and heavy metals were used as quality standards for herbal drugs and preparations. All values of LG remedy and twenty herb ingredients were showed in Tables 3, 4 and 5.

3.2.1 The Quality control of LG remedy

The study found that LG remedy met the standard guidelines of loss on drying, total ash, acid insoluble ash and heavy metal contamination as follows; the total ash, acid insoluble ash, and loss on drying was less than 10%, 2%, and 10%, respectively.^{6,7,8} Extractive value of water extracts of LG remedy was higher than ethanol extractive value. Arsenic (As), lead (Pb) and cadmium (Cd) were less than 4 ppm, 10ppm, and 0.3 ppm, respectively.

3.2.2 The Quality control of twenty herb ingredients

3.2.2.1 Loss on drying

All of herbs in this study met the standard guidelines of loss of drying at less than 10% as shown

in Table 3. The percentage loss on drying of LG remedy and its plant ingredients was ranged from 5.17% to 9.97%. The maximum percentage of loss on drying was *A. vulgaris* (9.97 ± 0.42) and the minimum percentage of loss on drying was *S. aromaticum* (5.17 ± 0.05).

3.2.2.2 Total ash

The maximum percentage total ash of LG remedy and plants ingredient was *O. sanctum* with 13.71 ± 0.21 %w/w and the minimum percentage total ash was *P. nigrum* with 1.63 ± 0.03 %w/w as shown in Table 3.

3.2.2.3 Acid insoluble ash

The percentage acid insoluble ash of LG remedy and all plant ingredients were ranged from 0.01 to 1.71% w/w. The maximum percentage of acid insoluble ash was *Z. zerumbet* (1.71 ± 0.17) and the minimum percentage of acid insoluble ash was *C. hystrix* (0.01 ± 0.03) as shown in Table 3.

Table 3 Quality control of Leard-Ngam remedy and its plant ingredients.

Herb	Quality control					
	Loss on drying		Total ash		Acid insoluble ash	
	(%) \pm SEM	Limit (%)	(%) \pm SEM	Limit (%)	(%) \pm SEM	Limit (%)
Leard-Ngam remedy	9.817 \pm 0.32	<10	7.330 \pm 0.075	<10	0.259 \pm 0.031	<2
<i>Allium sativum</i>	9.474 \pm 0.30	<10	4.154 \pm 0.085	<2.5	0.039 \pm 0.026	<1
<i>Amomum xanthioides</i>	7.985 \pm 0.47	<10	6.773 \pm 0.026	<10	0.770 \pm 0.580	<2
<i>Artemisia vulgaris</i>	9.973 \pm 0.42	<10	12.177 \pm 0.043	<10	1.117 \pm 0.153	<2
<i>Boesenbergia rotunda</i>	8.618 \pm 0.17	<10	7.866 \pm 0.028	<6	0.710 \pm 0.013	<2
<i>Citrus aurantifolia</i>	8.232 \pm 0.26	<10	9.804 \pm 2.812	<10	0.161 \pm 0.118	<2
<i>Citrus hystrix</i>	5.408 \pm 0.09	<12	7.476 \pm 0.027	<9	0.002 \pm 0.032	<1
<i>Cymbopogon citratus</i>	7.988 \pm 0.83	<10	7.788 \pm 0.021	<10	0.732 \pm 0.192	<2
<i>Glycyrrhiza glabra</i>	8.112 \pm 0.15	<10	6.278 \pm 0.001	<10	0.080 \pm 0.024	<2
<i>Mentha cordifolia</i>	9.488 \pm 0.03	<10	9.079 \pm 0.066	<10	0.019 \pm 0.100	<2
<i>Myristica fragrans</i>	7.497 \pm 0.14	<10	2.103 \pm 0.023	<10	0.115 \pm 0.050	<2
<i>Ocimum sanctum</i>	9.217 \pm 0.31	<14	13.709 \pm 0.211	<10	0.043 \pm 0.038	<2
<i>Oroxylum indicum</i>	8.714 \pm 0.42	<10	10.480 \pm 0.048	<10	0.047 \pm 0.021	<2
<i>Piper nigrum</i>	9.858 \pm 0.10	<10	1.629 \pm 0.025	<4	0.253 \pm 0.071	<0.5
<i>Piper retrofractum</i>	7.075 \pm 0.17	<13	5.263 \pm 0.777	<7.5	0.019 \pm 0.001	<0.4
<i>Piper sarmentosum</i>	9.178 \pm 0.54	<10	11.069 \pm 0.059	<20	0.510 \pm 0.045	<7
<i>Plumbago indica</i>	8.434 \pm 0.05	<10	6.257 \pm 0.011	<10	1.203 \pm 0.003	<2
<i>Syzygium aromaticum</i>	5.173 \pm 0.05	<10	5.383 \pm 0.077	<10	0.046 \pm 0.027	<2
<i>Zingiber cassumunar</i>	9.738 \pm 0.11	<13	4.433 \pm 0.008	<9	0.501 \pm 0.002	<3
<i>Zingiber officinale</i>	8.912 \pm 0.14	<11	5.705 \pm 0.006	<10	0.177 \pm 0.090	<1
<i>Zingiber zerumbet</i>	7.025 \pm 0.66	<10	8.422 \pm 0.071	<10	1.712 \pm 0.174	<2

3.2.2.4 Heavy metals of LG remedy and its plant ingredients

All twenty ingredient herbs of LG remedy and its plant ingredients showed that arsenic, lead and cadmium as determined by atomic absorption

spectrometry were less than 4 ppm, 10 ppm and 0.3 ppm, respectively, as showed in Table 4.

LG remedy and its plants ingredients were qualified according to Thai herbal pharmacopoeia criteria

Table 4 Quality control of Leard-Ngam remedy and its plant ingredients using atomic absorption spectrometry.

Herb	Quality control		
	Atomic absorption (ppm) ± SEM		
	As (not more than 4 ppm)	Pb (not more than 10 ppm)	Cd (not more than 0.3 ppm)
Leard-Ngam remedy	ND	ND	ND
<i>Allium sativum</i> L.	ND	ND	ND
<i>Amomum xanthioides</i> Wall.	ND	ND	ND
<i>Artemisia vulgaris</i> L.	ND	ND	ND
<i>Boesenbergia rotunda</i> (L.) Mansf	ND	ND	ND
<i>Citrus aurantifolia</i> (Christm.) Swingle	ND	ND	ND
<i>Citrus hystrix</i> DC.	ND	ND	ND
<i>Cymbopogon citratus</i> (DC.)	ND	ND	ND
<i>Glycyrrhiza glabra</i> L.	ND	ND	ND
<i>Mentha cordifolia</i> Opiz.	ND	ND	ND
<i>Myristica fragrans</i> Houtt.	ND	ND	ND
<i>Ocimum sanctum</i> Linn.	ND	ND	ND
<i>Oroxylum indicum</i> Linn	ND	ND	ND
<i>Piper nigrum</i> Linn.	ND	ND	ND
<i>Piper retrofractum</i> Vahl	ND	ND	ND
<i>Piper sarmentosum</i> Roxb.ex Hunter	ND	ND	ND
<i>Plumbago indica</i>	ND	ND	0.292 ± 0.015
<i>Syzygium aromaticum</i> (L.)	ND	ND	ND
<i>Zingiber cassumunar</i> Roxb.	ND	ND	ND
<i>Zingiber officinale</i> Roscoe	ND	ND	ND
<i>Zingiber zerumbet</i> (L.) Sm.	ND	ND	0.003 ± 0.003

Note: ND is Not detected

3.2.2.5 Extractive value

The water extractive values of eighteen plant ingredients of LG remedy were higher than all ethanolic extracts except the ethanolic extracts of *M. fragrans* and *P. Nigrum*. *M. fragrans* contained the

highest ethanolic extractive value (4.4%). the highest percentage of water extractive value (15.35%) was *A. Sativum* (Table 5). On the other hand, all other herbs had the extractive value of ethanol and water extract less than the given values by THP 2019.

Table 5 Extractive value of Leard-Ngam remedy and its plant ingredient

Herbs	Solvent	Code	Extractive value (%)
Leard-Ngam remedy	95% Ethanol	LG95	1.33
	water	LGW	4.08
<i>Allium sativum</i>	95% Ethanol	ASE	0.19
	water	ASW	15.35
<i>Amomum xanthioides</i>	95% Ethanol	AXE	0.45
	water	AXW	1.52
<i>Artemisia vulgaris</i>	95% Ethanol	AVE	0.78
	water	AVW	3.72
<i>Boesenbergia rotunda</i>	95% Ethanol	BRE	0.84
	water	BRW	2.46
<i>Citrus aurantifolia</i>	95% Ethanol	CAE	1.43
	water	CAW	5.47
<i>Citrus hystrix</i>	95% Ethanol	CHE	1.85
	water	CHA	5.65
<i>Cymbopogon citratus</i>	95% Ethanol	CCE	2.53
	water	CCW	4.71
<i>Glycyrrhiza glabra</i>	95% Ethanol	GGE	0.98
	water	GGW	2.97
<i>Mentha cordifolia</i>	95% Ethanol	MCE	0.83
	water	MCW	4.54
<i>Myristica fragrans</i>	95% Ethanol	MFE	4.44
	water	MFW	1.32
<i>Ocimum sanctum</i>	95% Ethanol	OSE	1.38
	water	OSW	4.07
<i>Oroxylum indicum</i>	95% Ethanol	OIE	0.76
	water	OIW	4.43
<i>Piper nigrum</i>	95% Ethanol	PNE	1.15
	water	PNW	0.40
<i>Piper retrofractum</i>	95% Ethanol	PRE	2.60
	water	PRW	4.58
<i>Piper sarmentosum</i>	95% Ethanol	PSE	0.55
	water	PSW	3.40
<i>Plumbago indica</i>	95% Ethanol	PIE	1.24
	water	PIW	5.57
<i>Syzygium aromaticum</i>	95% Ethanol	SAE	1.18
	water	SAW	5.10
<i>Zingiber cassumunar</i>	95% Ethanol	ZCE	1.54
	water	ZCW	3.20
<i>Zingiber officinale</i>	95% Ethanol	ZOE	0.68
	water	ZOW	3.28
<i>Zingiber zerumbet</i>	95% Ethanol	ZZE	0.57
	water	ZZW	2.94

Discussion

LG remedy is present in National List of Essential Medicine, 2018 and recommended to use for relieving uterine contraction during menstrual period (dysmenorrhea). The present study showed that the eugenol, austrobailignan, acetueugenol and piperine in LG remedy were detectable in other medicinal plants containing in LG remedy. Eugenol, acetueugenol were detected in *S. Aromaticum*.⁹ Piperine was found in *P. nigrum*, *P. retrofractum*, *P. Sarmentosum*. Austrobailignan was found in *M. fragrans*. Eugenol and piperine in previous study showed anti-inflammatory activity, especially COX-2 inhibitory activity, one of the causes of primary dysmenorrhea.^{10, 11, 12} In addition, eugenol and acetyleugenol exhibited DPPH radical scavenging activity.¹¹ Piperine, marker of LG remedy has potential to reduce nitric oxide^{13, 14}, and reduced COX-2 production.¹²

In addition, the present study showed the quality control of LG remedy was acceptable according to the standard guidelines of Thai Herbal Pharmacopoeia; loss on drying, total ash, and acid insoluble ash. However, some medicinal plants of LG remedy were below standard; firstly, *A. sativum* and *A. vulgaris* did not meet standard of total ash, possibly due to calcium oxalate, inorganic salt in these plants.^{15, 16, 17} *B. rotunda* using from rhizome and *O. sanctum* from root, the contamination of gravels and rocks from soil were judged to be caused by falling in standardization. LG remedy and twenty herbs passed the standard guidelines of heavy metal such as Lead, arsenic and cadmium according to Thai Herbal Pharmacopoeia. In conclusion, phytochemical compound of LG remedy were eugenol and piperine presented the inhibitory activity of COX-2 production which caused of primary dysmenorrhea as indicated in previous report.¹⁰ Some single herbs containing in LG remedy were out of standard. However, the combination of those herbs as a remedy, was overall qualified with standard guidelines of Thai Herbal Pharmacopoeia.

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บทคัดย่อ

มาตรฐานยาสมุนไพรและการวิเคราะห์สารพฤกษเคมีของตำรับยาเลื่อดงามและสมุนไพรเดี่ยวในตำรับ

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- บทนำ:** ตำรับยาเลื่อดงามเป็นตำรับยาใช้ในการลดอาการปวดประจำเดือนชนิดปฐมภูมิ และปรับสมดุลสุขภาพสตรี ประกอบไปด้วยสมุนไพร 20 ชนิด จึงควรมีการควบคุมมาตรฐานยา และศึกษาสารพฤกษเคมีเพื่อเป็นมาตรฐานในการนำไปใช้ต่อไป
- วัตถุประสงค์:** เพื่อวิเคราะห์หาสารพฤกษเคมีและตรวจมาตรฐานสมุนไพรของตำรับยา
- วิธีการศึกษา:** วิเคราะห์หาสารพฤกษเคมีด้วยเทคนิคแก๊สโครมาโทกราฟีเพื่อดูปริมาณ และชนิดของสารพฤกษเคมี ที่อยู่ในตำรับยาเลื่อดงาม และตรวจวัดมาตรฐานสมุนไพรของตำรับยาเลื่อดงาม ด้วย การศึกษา ปริมาณความชื้น ปริมาณเถ้า ปริมาณเถ้าที่ไม่ละลายในกรด การวิเคราะห์หาปริมาณอะตอมด้วยเครื่องอะตอมมิกแอบซอร์พชัน สเปกโทรโฟโตมิเตอร์เพื่อดูปริมาณสารหนู ตะกั่ว แคดเมียม
- ผลการศึกษา:** พบว่าตำรับยาเลื่อดงามผ่านมาตรฐานทุกการทดสอบ ส่วนสมุนไพรเดี่ยวพบว่า กระเทียม โกฐจุฬาลัมพา มีปริมาณเถ้าเกินมาตรฐาน อาจเนื่องจากมีปริมาณแคลเซียมออกไซด์มาก ส่วนกระชายและกะเพรา มีปริมาณเถ้าเกินเนื่องจาก ใช้ส่วนรากในการเตรียมยาจึงเป็นไปได้ว่าอาจมีการปนเปื้อนของหิน ดิน ทราาย เจือปนอยู่ แต่เมื่อนำไปวิเคราะห์หาปริมาณโลหะหนักในสมุนไพร ได้แก่ สารหนู ตะกั่ว และแคดเมียม พบว่าสมุนไพรทั้ง 20 ชนิดในตำรับยาเลื่อดงามมีค่าอยู่เกณฑ์มาตรฐาน และสารพฤกษเคมีที่พบในตำรับยาเลื่อดงาม ได้แก่ Eugenol, Austrobailignan, Aceteugenol และ Piperine
- สรุปผลการศึกษา:** สารพฤกษเคมีที่พบใน ตำรับยาเลื่อดงามคือสาร Eugenol, austrobailignan, aceteugenol and piperine. สมุนไพรบางตัวที่เป็นส่วนประกอบในตำรับยาเลื่อดงาม มีค่าบางค่าเกินเกณฑ์มาตรฐาน แต่เมื่อรวมเป็นตำรับแล้วพบว่าผ่านเกณฑ์มาตรฐานทุกการทดสอบ
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