Original article

Quality control standard values and stability study of ethanolic Ya-Hom KAE-LOM-WING-WIEN remedy extract on nitric oxide inhibition in LPS-stimulated RAW 264.7 macrophage cells of its anti-inflammatory activity

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Abstract

Introduction: Ya-Hom KAE-LOM-WING-WIEN remedy (KLWW) is included in the Thai National List of Essential Medicines A.D. 2013 and used for treating dizziness, fatigue, and sleeplessness. Quality control of plant ingredients and stability test of this remedy extract were ensured and controlled the quality of herbal medicines. In addition, standard quality values of this remedy and its plant ingredients have never been reported. The stability of this remedy extracts had also no report. The aim of this study was to investigate the quality control and stability test of KLWW on anti-inflammatory activity.

Method: Quality assessment method of remedy and its plant ingredients were determined by loss on drying, extractive value, total ash and acid insoluble ash, following Thai Herbal Pharmacopeia (THP) method. The stability testing of KLWW extract was stored over a six-month period, under 40 ± 2 °C with 75 ± 5% RH as accelerated conditions and also evaluated the anti-inflammatory activity by nitric oxide (NO) inhibitory assay in LPS-stimulated RAW 264.7 macrophage cells.

Results: The KLWW showed loss on drying value of 6.40 ± 0.20%, total ash value of 6.77 ± 0.48%, acid insoluble ash value of 0.91 ± 0.11%, and the ethanol and water-soluble extractive values of 7.64 ± 0.33 and 18.37 ± 0.74%, respectively. Stability test of the 95% ethanolic extract of KLWW on NO inhibition at days 0, 15, 30, 60, 90, 120, 150 and 180 showed IC_{50} values of 19.38 ± 1.13, 28.43 ± 078, 29.06 ± 1.71, 34.02 ± 0.40, 33.82 ± 0.44, 34.66 ± 2.32, 34.13 ± 1.68 and 32.04 ± 2.32 µg/ml, respectively, compared with prednisolone (positive control) with IC_{50} value of 0.16 ± 0.01 µg/ml.For stability study, KLWW extract lost anti-inflammatory activity after kept 15 days and the activity did not decreased any further up to 6 months.

Discussion and Conclusion: KLWW passed the standard value of THP standard in terms of loss on drying, extractive value, total ash, and acid insoluble ash. For stability study, KLWW extract lost anti-inflammatory activity after keep 15 days but it had anti-inflammatory activity because IC_{50} was in range 28 - 34 µg/ml. Thus, KLWW extract are stable in anti-inflammatory activity within two years.

Key words: Ya-Hom KAE-LOM-WING-WIEN remedy, Anti-inflammatory, Nitric oxide inhibition, Quality control, Stability

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Introduction

Ya-Hom is a Thai traditional herbal remedy and has long been used for treatment of dizziness, fatigue, fainting, abdominal discomfort *etc*. in Thailand. The remedy is a cardiotonic agent and is usually used for adjustment of wind element affecting healthy circulation[1]. There are five sub-types of Ya-Hom remedy are registered on the Thai National List of Herbal Medicinal Products A.D. 2013 i.e. Tip-Osot, Tepajit, Navakote, Kae-Lom-Wing-Wien and Intajak scriptures.

Ya-Hom KAE-LOM-WING-WIEN remedy (KLWW) has been used for treatment of dizziness, fatigue and sleeplessness[2]. The KLWW is nice-smelling herbal remedy and consists of various proportions of twenty-three medicinal ingredients[2]. There are *Glycyrrhiza glabra* L. (root), *Myristica fragrans* Houtt. (heartwood), *Syzygium aromaticum* Merr. et Perry (flower-bud), *Angelica sinensis* (Oliv.) Diels (root), *Ligusticum sinense* Oliv. cv.Chuanxiong Hort (rhizome), *Vetiveria zizanioides* (L.) Nash ex Small (root), *Nelumbo nucifera* Gaertn. (pollen), *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (bark), *Cinnamomum loureirii* Nees. (bark), *Cinnamomum verum* J. Presl. (bark), *Aquilaria crassna* Pierre ex Lecomte. (wood), *Euphorbia antiquorum* L. (heartwood), *Artemisia annua* L. (aerial part), *Terminalia chebula* Retz. var chebula (gall), *Alyxia reinwardtii* Blume var. lucida Markgr. (bark), borneo camphor, *Mimusops elengi* L. (wood), *Mesua ferrea* Linn. (flower), *Mimusops elengi* L. (flower), *Mammea siamensis* Kosterm (flower), *Ecdysanthera rosea* Hook. & Arn. (vine), sodium borate and *Dracaena loureiri* Gagnep. (heartwood).

Standard values of quality control on all Thai remedy and its plant ingredients were determined for quality controlling of the raw materials by following Thai Herbal Pharmacopoeia standard (THP)[3]. Anti-inflammatory activity by nitric oxide (NO) inhibitory assay had been studied on *Glycyrrhiza glabra* L.[4], *Myristica fragrans* Houtt.[5], *Angelica sinensis* (Oliv.) Diels[6], *Ligusticum* sinense Oliv. cv.Chuanxiong Hort[7], Vetiveria zizanioides (L.) Nash ex Small[8] , *Nelumbo nucifera* Gaertn.[9], *Cinnamomum verum* J. Presl.[10], *Mesua ferrea* Linn.[11], and *Mammea siamensis* Kosterm[6]. The stability test of the remedy extract implied that the extract had effective assurance activity. Therefore, this study was to determine standard values of quality control methods of this remedy and its plants ingredients and also investigated its stability test of KLWW on accelerated condition for assessing quality and effective assurance activity of this remedy.

Method

Chemicals

RPMI medium 1640 (RPMI 1640), Penicillin-Streptomycin (P/S), trypsin-ethylene diamine tetra acetic acid (EDTA) and trypan blue were purchased from Gibco, USA. Fetal Bovine Serum (FBS) was purchased from Biochem, Germany. Phosphate buffered saline (PBS) was purchased from Ameresco, USA. Sodium bicarbonate ($NaHCO_3$) was purchased from BHD, England. Lipopolysaccharide (LPS, from *Escherichia coli*) and 3-[4, 5-Dimethyl-2-thiazolyl]-2, 5-dipheyl-2H-tetrazalium bromide (MTT) were purchased from Sigma-Aldrich Chemical Company, USA. Absolute ethanol was purchased from QREC, New Zealand. Others chemicals were purchased at analytical grade.

Medicinal ingredients in Ya-Hom KAE-LOM-WING-WIEN

Ya-Hom KAE-LOM-WING-WIEN remedy consists of 23 medicinal ingredients which were collected from various parts of Thailand and India, China, Vietnam, Indonesia, Australia, and America in 2016. These plants were identified by the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The places of collection, part of plant and voucher specimens are shown in Table 1.

Table 1 List of ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy

Species	Places for specimen collection	Voucher specimen number	Thai name	Part of plant used
A. annua	India	SKP051010101	Kot chula lampa	aerial part
A. sinensis	China	SKP199011901	Kot chiang	root
A. crassna	Nakhon Ratchasima	SKP193010301	Krit sana	wood
A. reinwardtii	Surin	SKP013011801	Cha lud	bark
Borneo camphor	China	-	Phim sen	-
C. bejolghota	Surin, Nakhon Si Thammarat	SKP096030201	Sa mul lawang	bark
C. loureirii	China, Vietnam	SKP096031201	Op choei ywan	bark
C. verum	Indonesia	SKP096032201	Op choei thet	bark
D. loureiri	Nakhon Ratchasima	SKP005041201	Chan daeng	stem
E. antiquorum	Nakhon Ratchasima	SKP071050101	Kra lumphak	stem
E. rosea	Nakhon Ratchasima	SKP013051801	Mwak daeng	vine
G. glabra	China	SKP072070701	Cha aim thet	root
L. sinense	China	SKP199121901	Kot hua bua	rhizome
M. ferrea	Phetchabun	SKP083130601	Bunnak	flower
M. elengi	Angthong, Singburi	SKP171130501	Phikul	flower
*M. elengi**	Nakhon Ratchasima	SKP171130501	Khon dok	wood
M. fragrans	Australia	SKP121130601	Chan thet	stem
M. siamensis	Ratchaburi, Singburi	SKP083131901	Saraphi	flower
N. nucifera	Nakhon Sawan	SKP125141401	Bua luang	pollen
S. aromaticum	Indonesia	SKP123190101	Kan phlu	flower
Sodium borate	America	-	Nam phra santoeng	-
T. chebula	India	SKP0459200301	Kot phung pla	gall
V. zizanioides	Maha Sarakham	SKP081222601	Faek hom	root

Standard values of quality control of KLWW and its plant ingredients[12]

Standard values of quality control of the KLWW and its ingredients were investigated following THP standard[12] including loss on drying, extractive value, total ash, and acid insoluble ash. All of plant ingredients were investigated quality control methods except borneo camphor and sodium borate.

Moisture contents

Moisture content or loss on drying was analyzed by electronic moisture analyzer (Scaltec, Model: SMO 01). 5 g of each sample was put into the moisture analyzer at 105 °C. The weight of the dried sample was displayed and moisture content was calculated by the formula.

Extractive value

The study includes ethanol and water-soluble extractive values. Dried plant powder (5 g) were macerated in 100 ml of 95% ethanol in Erlenmeyer flask with foil, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. After that, the extract was dried at 105°C until constant weight. The procedure for determination of water-soluble extractive value is similarly to the method

for ethanol-soluble extractive value but using 0.25% chloroform in water instead of ethanol. The percentage of ethanol and water-soluble extractive values were calculated.

Total ash contents

Weight of sample (2 g) in crucible was recorded and the crucible was burned using muffle furnace (Thomas Scientific, Model: P 330) at 450°C for 9 hours. The crucible was cooled in a dessicator and then put in the muffle furnace at 450°C for 5 hours and placed in the dessicator until cool down. This procedure was repeated until the weight was stable. From the final weight, % total ash was calculated compared with the weight of before burning.

Acid insoluble ash contents

This method was continued from the total ash content method. The total ash was added to twenty-five ml of 10% hydrochloric acid (HCl) and then boiled for 5 minutes. The ashless was taken on filter paper and then washed by distilled water until pH 7. The residual ash on filter paper was put in the crucible and burned in muffle furnace at 500°C for 9 hours. After burning, weight of the ash was measured until the weight was stable. Percentage of acid insoluble ash was calculated.

The stability test of KLWW extract[13, 14]

The 95% ethanolic extract of KLWW was stored for 180 days under 40 ± 2 °C with 75 ± 5% RH as accelerated conditions. Samples were taken at days 0 (control sample), 15, 30, 60, 90, 120, 150 and 180 and each sample were evaluated the anti-inflammatory activity by nitric oxide (NO) inhibitory assay.

Assay of nitric oxide production inhibitory effect[15, 16]

Inhibitory activity on NO production in RAW 264.7 murine macrophage cells was used to evaluate anti-inflammatory activity. The RAW 264.7 cells (ATTC TIB-71) were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% P/S stored at 37 °C in 5% CO_2 atmosphere with 95% humidity. The cells were washed with PBS according to 0.25% trypsin-EDTA and then added fresh medium. After that it was centrifuged at 1,500 rpm for 5 min, the supernatant was removed and replaced with 10 ml of fresh medium. Viable cells were counted by using trypan blue and seeded at the given final concentration of 1×10^5 cells/well in 96 well plates. After incubation for 24 hours, the medium was removed and added fresh medium with 100 µl containing 2 ng/ml of lipopolysaccharide (LPS) together with sample solution at various concentrations (1-100 µg/ml). The extracts were dissolved with DMSO and diluted in medium according to the desired concentration, and then added 100 µl/well. After incubation for 24 hours, NO production was determined by measuring the accumulation of nitrite in the supernatant by adding 100 µl/well of the Griess reagent consist of 1% sulfanilamide in 5% H_2SO_4 and 0.1% naphthalene diamine dihydrochloride). Cytotoxicity testing was also tested to ensure that NO production was not produced by destroying cell membrane (% cytotoxicity less than 30%) by using MTT colorimetric method. The absorbance was measured at 570 nm by a microplate reader (Power Wave XS, BioTek) and a half maximal inhibitory concentration (IC_{50}) value were determined by Prism software.

Statistical analysis

All data are mean ± standard error of mean (n=3). The data were analyzed with SPSS (License Authorization Wizard) statistical software using repeated measures ANOVA test and then Wilcoxon Signed Rank test for data with non-normality and Paired t-test for data with normality. A p-value of less than 0.05 was considered statistical significance.

Results

The plant ingredients data of KLWW included scientific name, places for specimen collection, voucher specimen number, Thai name and part used were shown in Table 1. The results of standard values of quality control of KLWW and its plant ingredients were shown in Table 2. The stability of the 95% ethanolic extract of KLWW on anti-inflammatory activity through inhibitory activity of NO release were shown in Table 3.

Table 2 The results of quality control of KLWW and its plant ingredients

Sample	%Loss on drying	%Extractive value		%Ash content	
		Ethanol-soluble	Water-soluble	Total ash	Acid insoluble ash
A. annua	8.08 ± 0.23	5.10 ± 0.25	19.09 ± 0.24	13.18 ± 0.15	0.43 ± 0.01
A. sinensis	3.47 ± 0.29* (not more than 14%)	6.36 ± 0.62* (not less than 12%)	48.56 ± 4.47* (not less than 52%)	8.65 ± 0.37* (not more than 7%)	2.44 ± 0.34* (not more than 2%)
A. crassna	6.92 ± 0.10	3.62 ± 0.14	2.74 ± 0.02	0.74 ± 0.01	0.08 ± 0.01
A. reinwardtii	6.30 ± 0.34	1.28 ± 0.09	15.99 ± 0.23	8.20 ± 0.17	0.08 ± 0.02
C. bejolghota	8.01 ± 0.35	20.37 ± 2.37	13.48 ± 0.22	1.30 ± 0.02	0.06 ± 0.00
C. loureirii	6.27 ± 0.50	2.35 ± 0.25	5.89 ± 0.09	2.98 ± 0.02	0.09 ± 0.01
C. verum	4.47 ± 0.32	2.47 ± 0.34	9.65 ± 0.20	4.10 ± 0.07	0.06 ± 0.00
D. loureiri	4.40 ± 0.22** (not more than 8%)	18.73 ± 0.18** (not less than 12%)	2.61 ± 1.04** (not less than 1%)	5.62 ± 0.23** (not more than 1%)	0.37 ± 0.08 -
E. antiquorum	4.53 ± 0.18	24.02 ± 1.25	2.05 ± 0.21	4.20 ± 0.10	0.12 ± 0.01
E. rosea	5.81 ± 0.28	2.43 ± 0.30	10.14 ± 0.80	4.14 ± 0.38	0.14 ± 0.03
G. glabra	6.73 ± 0.62	6.99 ± 0.41	16.33 ± 0.50	8.83 ± 0.39	1.32 ± 0.18
L. sinense	8.68 ± 0.43	9.71 ± 0.57	21.61 ± 2.66	4.20 ± 0.12	0.36 ± 0.01
M. ferrea	7.13 ± 0.22** (not more than 11%)	15.77 ± 0.53** (not less than 4.5%)	9.39 ± 1.21** (not less than 2.5%)	4.59 ± 0.35** (not more than 5%)	1.54 ± 0.12** (not more than 1.5%)
M. elengi	8.72 ± 0.38** (not more than 16%)	8.06 ± 0.17** (not less than 8%)	11.96 ± 0.05 -	5.67 ± 0.23** (not more than 7%)	1.12 ± 0.18** (not more than 3%)
*M. elengi**	5.68 ± 0.21	5.02 ± 0.04	5.09 ± 0.02	1.02 ± 0.03	0.26 ± 0.01
M. fragrans	6.48 ± 0.44	6.61 ± 0.34	1.52 ± 0.04	7.78 ± 0.02	1.68 ± 1.08
M. siamensis	8.01 ± 0.20	15.08 ± 0.20	25.43 ± 0.31	8.12 ± 0.05	0.39 ± 0.01
N. nucifera	7.82 ± 0.29** (not more than 12%)	6.05 ± 0.07 -	7.62 ± 0.55** (not less than 10.5%)	7.89 ± 0.18** (not more than 6%)	1.77 ± 0.02** (not more than 1%)
S. aromaticum	5.78 ± 0.49	6.43 ± 0.16	20.74 ± 0.44	5.71 ± 0.13	0.19 ± 0.01
T. chebula	5.23 ± 0.37	21.56 ± 2.05	45.14 ± 0.82	3.72 ± 0.10	0.08 ± 0.03
V. zizanioides	7.91 ± 0.44	10.21 ± 0.51	40.10 ± 0.24	9.54 ± 0.07	1.85 ± 0.07
KLWW	6.40 ± 0.12	7.64 ± 0.33	18.37 ± 0.74	6.77 ± 0.48	0.91 ± 0.11

Note: * indicated the standard value of THP 2016,

** indicated the standard value of Vol.4 2014,

- indicated not reported

Table 3 Stability of the 95% ethanolic extract of KLWW by anti-inflammatory activity through inhibition of NO production (IC_{50} µg/ml ± SEM), (n=3)

Storage time of extract	Stability of 95% ethanolic extract of Ya-Hom remedy by inhibition nitric oxide production IC_{50} ± SEM (µg/ml)	Comparison of the p-value with Day 0
Day 0	19.38 ± 1.13	-
Day 15	28.43 ± 0.78	0.04
Day 30	29.06 ± 1.71	0.02
Day 60	34.02 ± 0.40	0.00
Day 90	33.82 ± 0.44	0.00
Day 120	34.66 ± 2.32	0.00
Day 150	34.13 ± 1.68	0.00
Day 180	32.04 ± 2.32	0.00

Note: significant at p-value < 0.05

The standard values of quality control of KLWW was found that the percentage loss on drying of KLWW was 6.40% ± 0.12%.The highest percentage of loss on drying was *Mimusops elengi* Linn. (8.72 ± 0.37%) and the lowest percentage of loss on drying was *Angelica sinensis* (Oliv.) Diels (3.47 ± 0.29%). The percentages of ethanol and water-soluble extractive values of KLWW were 7.64 ± 0.33% and 18.37 ± 0.74%, respectively. The highest percentage of ethanol-soluble extractive value was *Euphorbia antiquorum* L. (24.02 ± 1.25%) and the lowest percentage of ethanol-soluble extractive value was *Alyxia reinwardtii* Blume var. lucida Markgr. (1.28 ± 0.09%). The highest percentage of water-soluble extractive value was *Angelica sinensis* (Oliv.) Diels (48.56 ± 4.47%) and the lowest percentage of water-soluble extractive value was *Myristica fragrans* Houtt. (1.52 ± 0.04%). The percentage total ash of KLWW was 6.77 ± 0.48%. The highest percentage of total ash was *Artemisia annua* L. (13.18 ± 0.15 %) and the lowest percentage of total ash was *Aquilaria crassna* Pierre ex Lecomte. (0.74 ± 0.01%). The percentage acid insoluble ash of KLWW was 0.91 ± 0.11%. The highest percentage of acid insoluble ash was *Angelica sinensis* (Oliv.) Diels (2.44 ± 0.34 %) and the lowest percentage of acid insoluble ash was *Cinnamomum bejolghota* (Buch.-Ham.) Sweet and *Cinnamomum verum* J. Presl. (0.06 ± 0.00% and 0.06 ± 0.00%, respectively). The KLWW passed the standard criteria of THP[12].

The stability results of the 95% ethanolic KLWW extract on inhibited NO production at days 0, 15, 30, 60, 90, 120, 150 and 180 presented IC_{50} values of 19.38 ± 1.13, 28.43 ± 078, 29.06 ± 1.71, 34.02 ± 0.40, 33.82 ± 0.44, 34.66 ± 2.32, 34.13 ± 1.68, and 32.04 ± 2.32 µg/ml, respectively.

Discussion and Conclusion

The KLWW remedy is mostly used for adjustment of wind element for healthy circulation. The study of KLWW based on the Thai National List of Herbal Medicinal Products and control of quality ingredients in relation to the use of herbal medicines. Quality control methods and stability test of KLWW were determined. These results were the first study that presented the stability of ethanolic KLWW extract on NO inhibition in LPS-induced macrophage cells.

After keep for 180 days, the extract at day 180 could reduce NO release although it was different from the fresh extract (day 0). For stability study, KLWW extract lost anti-inflammatory activity after kept 15 days but it had anti-inflammatory activity because IC_{50} were in range 28 - 34 µg/ml after 15 days until 180 days compared with day 0 (IC_{50} as 19.38 µg/ml). Thus, it was concluded that KLWW extract is nearly stable on anti-inflammatory activity when kept in a closed container protected from light and stored at room temperature for at least two years[13, 14]. Although, the results were reduction of activity, KLWW extract was still effective anti-inflammatory activity.

The standard quality value of THP represented by loss on drying is not more than 10%. KLWW and its plant ingredients were within the standard value. The highest percentage of loss on drying was *Mimusops elengi* Linn. because this plant part is flower so it should be the highest moisture content because preparing of this flower cannot dry by high temperature. The lowest percentage of loss on drying was *Angelica sinensis* (Oliv.) Diels because this plant part is rhizome and export from China, so it should be made dryness for protection of microbial contamination. The highest percentage of total ash was *Artemisia annua* L. (13.18 ± 0.15 %) which is high value and did not pass of criteria of THP which ash content of the plant is not more than 10%. Aerial part of *Artemisia annua* L. which bought from India may contaminate from sand and small gravel from preparing plant. Its acid insoluble ash value is used for confirmation because this value is less than 0.5% (0.43%). If this value also showed higher than 2%, it is described that this plant was contaminated from heavy metal[12]. Thus, these results showed that the source of raw material for preparing traditional drug is necessary for standard values of plants. Although the percentage of total ash of Artemisia annua L., *Angelica sinensis* (Oliv.), *Dracaena loureiri* Gagnep., and *Nelumbo nucifera* Gaertn. did not pass of criteria, KLWW was within the standard value of THP (≤10%)[12]. The lowest percentage of total ash was *Aquilaria crassna* Pierre ex Lecomte. (0.74 ± 0.01%). This plant is wood part so it can be burned and no contaminated from sand. Acid insoluble ash of this plant also related with total ash content because it showed small value amount (0.08 ± 0.01%). The percentage of acid insoluble ash of KLWW was within the standard value of THP (≤ 2%) although *Angelica sinensis* (Oliv.) Diels and *Nelumbo nucifera* Gaertn. did not pass the standard value criteria of ≤ 2% and ≤ 1%, respectively. KLWW showed the percentage of water-soluble extractive value more than ethanol-soluble extractive value. *Angelica sinensis (Oliv.)* Diels and Nelumbo nucifera Gaertn. showed the percentage of ethanol and water-soluble extractive values less than the standard value of THP. These plants related with high total ash and acid insoluble ash contents.

In conclusion, the results indicated that the KLWW extract can reduce inflammation through NO inhibition and stable within 2 years. The standard value of plant ingredients of KLWW should be benefit for quality control of raw materials of plant ingredients and be guided for preparing KLWW. However, KLWW extract should be isolated active anti-inflammatory compounds and testing in animal model.

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บทคัดย่อ

การควบคุมคุณภาพ และการศึกษาความคงตัวของสารสกัดชั้นเอทานอลของตำรับยาหอมแก้ลมวิงเวียนในการยับยั้งไนตริกออกไซด์ในเซลล์แมคโครฟาจ RAW 264.7 ที่กระตุ้นด้วย LPS

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บทนำ: ตำรับยาหอมแก้ลมวิงเวียนเป็นตำรับที่อยู่ในบัญชียาหลักแห่งชาติ พ.ศ.๒๕๕๖ และใช้รักษาแก้ลมวิงเวียน, อ่อนเพลีย และนอนไม่หลับ การควบคุมคุณภาพวัตถุดิบสมุนไพรในตำรับและการทดสอบความคงตัวของสารสกัดตำรับส่งผลต่อความมั่นใจและควบคุมคุณภาพของยาสมุนไพร นอกจากนี้ มาตรฐานการควบคุมคุณภาพของตำรับและสมุนไพรในตำรับนี้ยังไม่เคยมีการรายงาน รวมถึงการทดสอบความคงตัวของตำรับ วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาการควบคุมคุณภาพและความคงตัวของตำรับโดยนำมาทดสอบฤทธิ์ต้านการอักเสบ

วิธีการศึกษา: การทดสอบควบคุมคุณภาพของตำรับและสมุนไพรในตำรับประกอบด้วย ปริมาณความชื้น ปริมาณสารสกัด ปริมาณเถ้ารวม และปริมาณเถ้าที่ไม่ละลายในกรดตามวิธีตำรามาตรฐานยาสมุนไพรไทย การทดสอบความคงตัวของสารสกัดตำรับโดยเก็บเป็นระยะเวลา ๖ เดือน ภายใต้สภาวะเร่งที่อุณหภูมิ 40 ± 2 °C และความชื้นสัมพัทธ์ 75 ± 5% โดยนำมาทดสอบฤทธิ์ต้านการอักเสบโดยวิธี NO inhibitory assay ในเซลล์แมคโครฟาจ RAW 264.7 ที่กระตุ้นด้วย LPS

ผลการศึกษา: ตำรับมีปริมาณความชื้น เท่ากับ 6.40 ± 0.20%, ปริมาณเถ้ารวม เท่ากับ 6.77 ± 0.48%, ปริมาณเถ้าที่ไม่ละลายในกรด เท่ากับ 0.91 ± 0.11%, ปริมาณสารสกัด ของชั้นเอทานอลและชั้นน้ำ เท่ากับ 7.64 ± 0.33 และ 18.37 ± 0.74% ตามลำดับ การทดสอบความคงตัวของสารสกัดตำรับชั้น 95% เอทานอลในวันที่ ๐, ๑๕, ๓๐, ๖๐, ๙๐, ๑๒๐, ๑๕๐ และ ๑๘๐ พบว่ามีค่า IC_{50} ดังนี้ 19.38 ± 1.13, 28.43 ± 078, 29.06 ± 1.71, 34.02 ± 0.40, 33.82 ± 0.44, 34.66 ± 2.32, 34.13 ± 1.68 และ 32.04 ± 2.32 ไมโครกรัม/มิลลิลิตร ตามลำดับ โดยเปรียบเทียบกับเพรดนิโซโลน (กลุ่มควบคุมแบบบวก) โดยมีค่า IC_{50} เท่ากับ ๐.๑๖ ± ๐.๐๑ ไมโครกรัม/มิลลิลิตร ส่วนการศึกษาความคงตัวของสารสกัดตำรับพบว่าแม้ฤทธิ์ต้านการอักเสบจะลดลงหลังจากผ่านไป ๑๕ วัน และพบว่าฤทธิ์ไม่ลดลงอีกต่อเนื่องนานถึง ๖ เดือน

วิจารณ์ และสรุปผลการศึกษา: ตำรับแก้ลมวิงเวียนผ่านการทดสอบการควบคุมคุณภาพตามตำรามาตรฐานยาสมุนไพรไทย ประกอบด้วย ปริมาณ ความชื้น ปริมาณสารสกัด ปริมาณเถ้ารวม และปริมาณเถ้าที่ไม่ละลายในกรด ส่วนการทดสอบความคงตัวของสารสกัดตำรับเมื่อทดสอบฤทธิ์ต้านการอักเสบพบว่าฤทธิ์มีค่าลดลงหลังผ่านไป ๑๕ วัน แต่ยังคงมีฤทธิ์ต้านการอักเสบเนื่องจากค่า IC_{50} อยู่ในช่วง ๒๘ - ๓๔ ไมโครกรัม/มิลลิลิตร ดังนั้น สารสกัดตำรับยาหอมแก้ลมวิงเวียนยังคงฤทธิ์ต้านการอักเสบได้ภายใน ๒ ปี

คำสำคัญ: ตำรับยาหอมแก้ลมวิงเวียน, ฤทธิ์ต้านการอักเสบ, การยับยั้งไนตริกออกไซด์, การควบคุมคุณภาพ, การทดสอบความคงตัว