Standardization of *Leonurus sibiricus* L. aerial part and capillary electrophoresis quantitative analysis of its leonurine content

**Abstract**

The quality parameters of *Leonurus sibiricus* L. aerial part crude drugs were evaluated. Fifteen crude drugs were collected from various locations throughout Thailand. The transverse section of the stem of *L. sibiricus* showed quadrangular character highlighted the ribs with angular collenchyma. The epidermis was uniseriate with abundant glandular trichomes distribution. Prismatic calcium oxalate prisms were found in the stem medullary parenchyma. The histological character of crude drug powder showed bordered pitted vessel, fragment of fiber, glandular trichome, prism crystal, spiral vessel, starch granule, and stomata. The loss on drying, total ash, acid‑insoluble ash, and moisture contents should be not more than 8.18, 15.28, 4.04, and 8.91 g/100 g dry weight, whereas ethanol and water‑soluble extractive values should be not less than 7.67, and 17.21 g/100 g of dry weight, respectively. Leonurine in the crude drugs were analyzed by capillary electrophoresis (CE) with photodiode array detector. The ethanolic extraction performed by Soxhlet apparatus yielded 18.86 ± 4.09 g/100 g dry weight. The electropherogram detected at 277 nm showed the migration time of leonurine at 6.2 min. The developed CE was found to be valid for leonurine quantification in *L. sibiricus* ethanolic extract. The contents of leonurine in 15 crude drugs ranged from 0.79 to 4.23 mg/g with the average of 2.38 ± 1.10 mg/g dry weight. This study established the pharmacognostic specification of *L. sibiricus* crude drug in Thailand with special reference to a bioactive compound, leonurine. CE was beneficial technique for the analysis of leonurine in *L. sibiricus* aerial parts.

**Key words:** Capillary electrophoresis, leonurine, *Leonurus sibiricus* L., standardization

**INTRODUCTION**

*Leonurus sibiricus* L. or Siberian motherwort, locally known in Thailand as “Khanchaa thet” or “Saa saa, Saa nam,” is an herbaceous plant belonging to the Labiatae family. It is an annual or biennial herb with stems erect, three lobes pinnately divided leaves, lobules of leaves 1–3 mm wide, flower sessile, corolla white or reddish to purple-red, calyx densely pilose, especially at the middle. *L. sibiricus* is confined to stony or sandy grasslands and *Pinus* forests in its original native distribution (Russia, Mongolia, and China).[1] *L. sibiricus* has been used in traditional Thai herbal medicine, especially in women remedies for menstrual induction, preventing postpartum hemorrhage and improving blood circulation.

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The pharmacological study of *L. sibiricus* showed that the ethanolic extract of leaves and stems had promising activity on uterine contraction in Wistar rats. In addition, *L. sibiricus* is one of the main ingredients in Prachomphothawee remedy, which is listed in Thailand National List of Essential Medicines (NLEM), for traditional treatment of common cold and allergic rhinitis symptoms. The clinical trials of this remedy on allergic rhinitis treatment revealed significant improvement of the symptoms and better quality of life related to rhinoconjunctivitis. Drugs listed in NLEM must be essential to prevent and resolve health problems, the quality of crude drug including the active chemical composition is essential for medicinal efficacy. Various types of chemical compounds were found in the aerial parts of *L. sibiricus*, for example, alkaloid (leonurine), labdane diterpenoid (sibiricinones), leoponpin, isoleoheterin, leosibiric acids, diterpene lactone (leonotinin, leonotin, dubiin, and nepetaefuran), flavonoid (genkwanin and rutin), polyphenol (chlorogenic acid and caffeic acid), iridoid glycoside (acetylhapargide and ajugoside), phenylpropanoid glycoside (lavandulifolioside and verbascoside), sterol (β-sitosterol and β-sitosterol glucoside), etc., Leonurine [Figure 1] is a highlighted compound in *L. sibiricus* regarding its pharmacological properties such as neuroprotective effects and cardiovascular protective effects. Leonurine or 4-guanidino-n-butyl syringate is a pseudoalkaloid which is acylguanidino derivative of syringic acid and 4-guanidino-n-butanol. To identify the active constituents in medicinal plants, chromatographic techniques are world-widely used in various studies. High performance liquid chromatography (HPLC) has been previously reported for the analysis of leonurine in *L. japonicus* Houtt. In addition, capillary electrophoresis (CE) is accepted as a separation technique for plant secondary metabolite analysis, for example, iridoids, phenylpropanoids, flavonoids, lignans, and alkaloids. CE use high-voltage direct current electric field as driving force for analyte separation performed in capillaries and the analysis time or migration time is usually <30 min, faster than HPLC. At present, leonurine quantification in *L. sibiricus* in Thailand, particularly with the CE technique, has not been reported. Therefore, this study presented the quality parameters of *L. sibiricus* aerial part crude drug in Thailand with special reference to its leonurine content analyzed by CE.

**MATERIALS AND METHODS**

**Sample collection**

*L. sibiricus* dried aerial parts were provided from 15 traditional drugstores located in four regions of Thailand [Table 1]. All crude drugs were authenticated by the expert taxonomist (Nijsiri Ruangrungsi) as well as comparison with the identified herbarium specimens at the Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Thailand. Microscopic anatomy of the stem was also compared to previous report. Voucher specimens and numbers were deposited at the research institution.

**Instruments and chemicals**

CE system (Agilent 7100, USA), muffle furnace (Carbolite Gero, UK), digital microscope (Carl Zeiss, Germany), water purification system (Heal Force, China), TLC silica gel 60 GF254 plate (Merck, Germany), leonurine (SML0670, Sigma-Aldrich, USA), and AR grade solvents (RCI Labscan, Thailand).

**Determination of pharmacognostic specification**

**Plant material identification**

*L. sibiricus* flowering branches were visually inspected. The transverse section of the stem was examined under a digital microscope for the anatomical characteristics. The crude drug was pulverized and microscopically investigated for histological characteristics. Those characters were botanically illustrated by hand drawing.

**Determination of loss on drying, total ash, and acid-insoluble ash**

Three grams of the powdered crude drug were spread in an even layer in a pre-weighed crucible and heated at 105°C for the loss on drying content. After that, the crucible was placed in a muffle furnace for incineration at 550°C for 6–8 h. The crucible containing the total ash was weighed after cooling in a desiccator. Then, 25 ml of hydrochloric acid (70 g/l) was added and boiled gently for 5 min. The insoluble matters were collected on an ashless filter paper and transferred to the original crucible, dried on a hot plate, and incinerated again. After cooling in a desiccator, the crucible containing the acid-insoluble ash was weighed.

**Determination of ethanol-and water-soluble extractive matters**

Five grams of the powdered crude drug was macerated in 70 ml of 95% ethanol or water for 6 h under shaking then 18 h under standing at room temperature. After filtration, the marc was washed and the volume was adjusted to 100 ml with the solvent used. Twenty milliliters of the filtrate were aliquoted into a pre-weighed small beaker, heated to dryness at 105°C, and weighed. The extractive matters were calculated.

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![Chemical structure of leonurine](image_url)

**Figure 1**: Chemical structure of leonurine

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**Table 1**: List of 15 traditional drugstores located in four regions of Thailand

| Region       | Drugstores |
|--------------|------------|
| North        | 4          |
| Northeast    | 4          |
| South        | 3          |
| Southeast    | 4          |

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**Table 2**: List of chemicals used in the study

| Chemical                  | Manufacturer/Grade |
|---------------------------|--------------------|
| HPLC system               | Agilent 7100, USA  |
| TLC silica gel 60 GF254   | Merck, Germany     |
| AR grade solvents         | RCI Labscan, Thailand |
| Digital microscope        | Carl Zeiss, Germany |
| Water purification system | Heal Force, China  |

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**Table 3**: Microscopic anatomy of the stem

| Characteristic              | Observation          |
|----------------------------|----------------------|
| Trachea number             | 1                    |
| Vascular bundle            | 4                    |
| Epidermal cell wall thickness | 15-20 µm          |

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**Table 4**: Pharmacognostic specification

| Specification               | Observation          |
|----------------------------|----------------------|
| Plant material identification | *L. sibiricus* flowering branches were visually inspected. |
| Determination of pharmacognostic specification | Plant material identification |
Determination of moisture content
Fifty grams of the powdered crude drug was transferred to the flask containing 200 ml of water-saturated toluene. The flask was connected to the apparatus for azeotropic distillation of water in crude drug and toluene. The volume of water in the receiving tube was then recorded.[26]

Thin-layer chromatographic fingerprint identification
Another one of 20-ml aliquot of the ethanolic filtrate from the extractive matter determination was evaporated and re-dissolved in 0.5 ml of methanol. Five microliters of the extract were applied to TLC plate, developed with the mobile phase of ethyl acetate: methanol: water: formic acid (8.4: 0.6: 0.5: 0.5) and observed under 254 and 366 nm ultraviolet light as well as by spraying with p-anisaldehyde’s reagent.

Quantitative analysis of leonurine by capillary electrophoresis
Plant extraction
Five grams of each cleaned, dried, and pulverized sample was exhaustively extracted with 95% ethanol (200 mL) by the Soxhlet apparatus (6 h). After filtration, the filtrate was evaporated to dryness. The extract was weighed to calculate the % yield and then dissolved in methanol to obtain the final concentration (1 mg/1 mL). The extract in methanol was diluted to various concentrations and filtered through a 0.45 µm PTFE membrane syringe filter. The standard stock solutions of leonurine (20-100 µg/mL) were applied in triplicate on a CE column. The resolved peak area was recorded for each of the standard concentration. The calibration curve of leonurine was plotted by taking peak area versus concentrations of standard.

Capillary electrophoresis conditions
CE system comprised of Agilent 7100 CE equipped with auto-sampler and photodiode array (PDA) detector. The condition was set at 20°C, and applied voltage of 25 kV. The detection was performed at 277 nm. All filtered samples and standard were injected 5 s from each vial. The buffer solution was 20 mM sodium phosphate buffer, pH 7.9. Capillary column was uncoated fused silica capillary 64.5 cm length (56 cm to the detector) × 50 µm i. d. The leonurine content of each sample was determined by comparing the peak area of standard leonurine with a calibration curve. All data acquisition was performed using HP ChemStation software, version B.04.03.

Method validation
According to ICH guidelines,[27] specificity, linearity, limit of detection and limit of quantification, precision, accuracy, and robustness were checked for confirming method validation. All the parameters were performed in triplicates.

Specificity
The specificity of the method was determined by analyzing the absorbance spectra of standard leonurine and samples. Peak purity was evaluated by comparing its peak at peak start, peak apex, and peakend position.
**Linearity**
The linearity of the method was expressed as a calibration range generated by plotting peak areas versus concentrations of standard leonurine. The coefficient of determination ($R^2$) was calculated using Excel software.

**Limit of detection and limit of quantification**
LOD, the lowest amount that can be detected, and LOQ, the lowest amount that can be quantitated, were calculated from the calibration curve using the following formula:

$$\text{LOD} = \frac{3.3 \times \text{(residual SD)}}{\text{S}}, \quad \text{LOQ} = \frac{10 \times \text{(residual SD)}}{\text{S}}$$

Where residual SD = the residual standard deviation of the regression line, $S$ = the slope of the regression line.

**Precision**
The repeatability and intermediate precision were assessed by analyzing the sample solution with three concentrations (low, medium, and high) (each in triplicate) on the same day and three different days, respectively. The precision of leonurine content analysis was determined in terms of percent relative standard deviation (% RSD) by the following equation:

$$\% \text{RSD} = \frac{\text{SD}}{\text{MEAN}} \times 100$$

**Accuracy**
The percent recovery was determined by adding different level concentrations of standard leonurine to a pre-analyzed sample. The analysis was done by the proposed CE method and the analysis was carried out in triplicate.

$$\% \text{Recovery} = \frac{A}{B + C} \times 100$$

Where $A$ = the amount of leonurine found in the spiked extracted sample

$B$ = the amount of leonurine found in the un-spiked extracted sample

$C$ = the amount of standard leonurine was added to the extracted sample

**Robustness**
A small variation on the pH buffer solution from 7.8 to 8.0 was applied for the robustness. The result was expressed as % RSD.

**RESULTS AND DISCUSSION**

**Pharmacognostic specification of Leonurus sibiricus aerial part**
The drawing of a flowering branch and photograph of the dried crude drug (aerial parts) of $L$. sibiricus are shown in Figures 2 and 3. The transverse section of the stem of $L$. sibiricus showed anatomical characters of collenchyma, epidermis, glandular trichome, parenchyma, prism crystal, xylem fiber, and xylem vessel [Figure 4]. The quadrangular stem highlighted the ribs with angular collenchymatous cells was illustrated. The epidermis was uniseriate with abundant glandular trichomes distribution. Prismatic calcium oxalate prisms were found in the stem medullary parenchyma. The microscopic anatomy of $L$. sibiricus stem in Thailand was found to be unique as $L$. sibiricus stem reported in Brazil.[25] The histological evaluation of powdered crude drug showed a bordered pitted vessel, fragment of fiber, fragment of the lower epidermis, glandular trichome, prism crystal, parenchyma in longitudinal view, parenchyma in transverse view, spiral vessel, starch granule, and stomata [Figure 5]. The physicochemical parameters due to the quality of $L$. sibiricus are shown in Table 2. The loss on drying, total ash, acid-insoluble ash, and moisture contents should be not more than 8.18, 15.28, 4.04, and 8.91% by dry weight, whereas ethanol and water-soluble extractive values should be not >7.67 and 17.21 g/100 g of dry weight, respectively. TLC fingerprint is shown in Figure 6. The total

![Figure 2: Leonurus sibiricus flowering branch](image1)

![Figure 3: Crude drug of Leonurus sibiricus aerial parts](image2)
and acid-insoluble ashes were higher than previous study of L. sibiricus crude drugs in Korea in 2001 which reported loss on drying, total ash, and acid-insoluble ash as 7.94% ±0.63%, 8.51% ±2.1%, and 1.24% ±0.77%, respectively.\cite{27}

Leonurine content in Leonurus sibiricus aerial part

The ethanolic extraction using Soxhlet apparatus of L. sibiricus crude drugs from 15 locations yielded 18.86 ± 4.09% dry weight [Table 1]. CE coupling with PDA detector was used for quantitative analysis of leonurine in the extracts in this study due to several advantages, i.e. high separation efficiency, short analysis times, low waste generation, and a diverse range of applications.\cite{29} The optimized condition was developed and the method was validated in terms of linearity, accuracy, precision, LOD, LOQ, specificity, and robustness [Table 3].\cite{27} The
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Table 2: The physicochemical parameter due to quality of Leonurus sibiricus

| Parameter                        | Content (percentage by dry weight)* |
|----------------------------------|-------------------------------------|
| Total ash                        | 15.28±0.16                          |
| Acid-insoluble ash               | 4.04±0.36                           |
| Ethanol-soluble extractive       | 7.67±0.21                           |
| Water-soluble extractive         | 17.21±1.20                          |
| Loss on drying                   | 8.18±0.08                           |
| Moisture                         | 8.91±0.65                           |

*The parameters were shown as grand mean±pooled SD. The samples were from 15 different sources. Each sample was performed in triplicate. SD: Standard deviation

Table 3: Method validation parameters of leonurine in Leonurus sibiricus extract

| Parameters                  | Validity |
|-----------------------------|----------|
| Linearity range (µg/mL)     | 20-100   |
| Coefficient of determination (R²) | 0.9995   |
| Linear regression equation (y) | 0.0871x+0.9537 |
| Precision: Repeatability (average % RSD) | 2.97     |
| Precision: Intermediate precision (average % RSD) | 3.16     |
| LOD (µg/mL)                 | 1.59     |
| LOQ (µg/mL)                 | 4.82     |
| Accuracy (average % recovery) | 99.54     |
| Robustness (average % RSD)  | 2.38     |

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

The electropherogram of the ethanolic extract of L. sibiricus is shown in Figure 7. Leonurine migration time was 6.2 min. The absorbance spectrum of leonurine scanned from 200 to 700 nm with the maximum absorption at 277 nm is shown in Figure 8. The identity of the spectra of standard and sample leonurine [Figure 8] as well as peak purity index of 0.9999 [Figure 9] represented the specificity of the method. The calibration curve showed linearity in the range of 20–100 µg/mL with the regression equation of y = 0.0871x + 0.9537 and the coefficient of determination (R²) was 0.9995 [Figure 10]. The accuracy was confirmed by spiking standard leonurine at three different concentrations (10, 30, and 50 µg/mL) into L. sibiricus extracted sample. The result was found to be 96.39%–101.83% recovery (average 99.54% recovery) [Table 3]. The average values of repeatability and intermediate precisions were 2.97 and 3.16% RSD, respectively. The limit of detection and the limit of quantitation, calculated from the residual standard deviation of the regression line and the slope of the calibration curve, displayed 1.59 and 4.82 µg/mL, respectively. The robustness was performed by varying the pH buffer solution from 7.8–8.0, and the average result was found to be 2.38% RSD [Table 3]. The developed CE was valid for the quantification of leonurine in L. sibiricus ethanolic extract. Table 1 shows the contents of leonurine in 15 L. sibiricus crude drugs that ranged from 0.79 to 4.23 mg/g with the average of 2.38 ± 1.10 mg/g or 0.24%±0.11%. The amount of leonurine in L. sibiricus crude drug in this study was higher than the previous report of L. sibiricus crude drug in Korea that demonstrated the leonurine content analyzed by HPLC as 0.124%±0.066%.[28] In addition, leonurine found in this study seemed to be higher than leonurine in L. japonicus crude drug in China quantitated by HPLC-MS/MS that was found to be 0.73 to 2.33 mg/g.[19] This finding revealed the beneficial application of CE technique for quality evaluation of medicinal plant material with reference to its bioactive phytochemicals.

CONCLUSION

The pharmacognostic specification of L. sibiricus in Thailand was established. The microscopic characteristics, including the transverse section of the stem and the powder characters of L. sibiricus, were illustrated. The TLC fingerprint and physicochemical parameters were demonstrated by this study and could be useful for the identification of L. sibiricus dried crude drug. The CE equipped with a PDA detector was developed, validated, and performed for quantification of leonurine in L. sibiricus. The leonurine contents in L. sibiricus from various areas in Thailand were revealed which could be used for the specification of this crude drug concerning its chemical marker.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Leonurus sibiricus Linnaeus. Flora of China 1994; 17:162–165. [cited 2021 Mar 26]. Available from: http://efloras.org/.
2. Kanchanapoo J, Kaewamatawong R, Khamdang S, Chinsai K,
Suwatronnakorn, et al.: Leonurus sibiricus aerial part: standardization and leonurine content

1. Ubolwat S, Jandee K. Effects of Thai herbs used in traditional women remedies on the uterine contraction. Thai Pharm Health Sci J 2011;6:202-8.

2. Jaroenyamprasater P, Ounprasertsuk J, Krutchangthong P, Dumklieng W. Herbal postpartum care in Thai traditional medicine. Proceedings of the International Academic Multidisciplinary Research Conference; 2019 Mar 7-8, London, pp 292-7.

3. Leonurus sibiricus (PROSEA). PlantUse English contributors; [updated 2016 Mar 11; cited 2021 Feb 22]. Available from: https://uses.plantnet-project.org/en/index.php?title=Leonurus_sibiricus_(PROSEA)&oldid=216970.

4. Leangpanich S, Itharat A, Chanvimalueng W, Mukkasombat N. A preliminary study on efficacy of Prapchompoothaweep remedy for treatment of allergic rhinitis patients and their quality of life after the treatment. Thammasat Med J. 2019;19:537-46.

5. Onthong N, Chonpatatip U, Rajanian Y, Patthananurak K, Sangvichien S, Kamoltham T. A comparative study on the effects of Prabchompoothaweep remedy and loratadine in treatment of patients with allergic rhinitis and upper respiratory tract infections at Pathumtani hospital. TH J of Health Edu. 2019;42:135-45.

6. Hayashi Y. Studies on the ingredients of Leonurus sibiricus L. II. Structure of leonurine. Yakugaku Zasshi. 1962;82:1025-7.

7. Zachow LL, Ávila JM, Saldanha GA, Mostardeiro MA, da Silva UF, Morel AF, et al. Chemical composition and evaluation of prolyl oligopeptidase and acetylcholinesterase inhibitory activities of Leonurus sibiricus L. from Brazil. Nat Prod Res. 2017;31:1459-63.

8. Boalino DM, McLean S, Reynolds WF, Tinto WF. Labdane diterpenes of Leonurus sibiricus. J Nat Prod. 2004;67:714-7.

9. Narukawa Y, Niimura A, Noguchi H, Tamura H, Kiuchi F. New diterpenoids with estrogen sulfotransferase inhibitory activity of Leonurus sibiricus. J Nat Prod. 2004;67:714-7.

10. Duarte MR, Lopes JF. Morfoanatomia Foliare e Caulinar de Leonurus sibiricus L. II. Acta Farm Bonaer. 2005;24:68-74.

11. Pitschmann A, Zehl M, Heiss E, Purevsuren S, Urban E, Dirsch VM, et al. Quantitation of phenylpropanoids and iridoids in insulin-sensitising extracts of Leonurus sibiricus L. (Lamiaceae). Phytochem Anal. 2016;27:23-31.

12. Sato M, Satoh Y, Isobe K, Fujimoto Y. Studies on the constituents of Leonurus sibiricus L. Chem Pharm Bull. 2003;51:341-2.

13. Lang D, Jia W, Zhu YZ. Leonurine, a potential agent of traditional Chinese medicine: recent updates and future perspectives. Nat Prod Commun. 2016;11:1757-61.

14. Zhu YZ, Wu W, Zhu Q, Liu X. Discovery of Leonurine and therapeutic applications: from bench to bedside. Pharmacol Ther. 2018;188:26-35.

15. Huang L, Xu DQ, Chen YY, Yue SJ, Tang YP. Leonurine, a potential drug for the treatment of cardiovascular system and central nervous system diseases. Brain Behav. 2021;11:e01995.

16. Yeung H, Kong Y, Lay W, Cheng K. The structure and biological effect of leonurine. Planta Med. 1977;31:51-6.

17. Tan YJ, Xu DQ, Yue SJ, Tang YP, Guo S, Yan H, et al. Comparative analysis of the main active constituents from different parts of Leonurus japonicus Houtt. and from different regions in China by ultra-high performance liquid chromatography with triple quadrupole tandem mass spectrometry. J Pharm Biomed Anal. 2020;177:112873.

18. Chen Z, Wu JB, Liao XJ, Yang W, Song K. Development and validation of an UPLC-DAD-MS method for the determination of leonurine in Chinese motherwort (Leonurus japonicus). J Chromatogr Sci. 2010;48:802-6.

19. Xie J, Sang L, Zhang Y, Fang L, Li Y. Determination of stachydrine and leonurine in Herba leonuri and its succedaneum—Herba lagopsis—with a sensitive HPLC–MS/MS method. J Liq Chromatogr Relat Technol. 2015;38:810-5.

20. Gomes AF, Ganzeria M, Schwaiger S, Stuppner H, Halabalaki M, Almeida MP, et al. Simultaneous determination of iridoids, phenylpropanoids and flavonoids in Lippia alba extracts by micellar electrokinetic capillary chromatography. Microchem J. 2018;138:494-500.

21. Liang J, Gong FQ, Sun HM. Simultaneous separation of eight lignans in Forsythia suspensa by β-cyclodextrin-modified capillary zone electrophoresis. Molecules. 2018;23:514.

22. Hou Z, Sun G, Guo Y, Yang F, Gong D. Capillary electrophoresis fingerprints combined with linear quantitative profiling method to monitor the quality consistency and predict the antioxidant activity of alkaloids of Sophora flavescens. J Chromatogr B. 2019;1133:121827.

23. Wang J, Jiang Y, Wang B, Zhang N. A review on analytical methods for natural berberine alkaloids. J Sep Sci. 2019;42(9):1794-815.

24. Liu X, Jiang W, Su M, Sun Y, Liu H, Nie L, et al. Quality evaluation of traditional Chinese medicines based on fingerprinting, J Sep Sci. 2020;43:6-17.

25. Duarte MR, Lopes JF. Morfoanatomia Foliare e Caulinar de Leonurus sibiricus L., Lamiaceae. Acta Farm Bonaer. 2005;24:68-74.

26. Pitakpawasutthi, Y, Palanuvej C, and Ruangrungsi N, Quality evaluation of Kaempferia parviflora rhizome with reference to 7,7-dimethoxyflavone. J Adv Pharm Technol Res. 2018;9:26–31.

27. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1). Geneva (switzerland); ICH; 2005.