Quantification of sinensetin in *Orthosiphon stamineus* from various phytogeographical zones in Indonesia

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**ARTICLE INFO**

Received on: 07/07/2022
Accepted on: 23/12/2022
Available Online: 04/03/2023

Key words: Marker, *Orthosiphon stamineus*, phytogeographical, sinensetin, TLC-densitometer.

**ABSTRACT**

*Orthosiphon stamineus* is widely used as an ingredient in traditional medicine and functional food partially for its main active compound, sinensetin. Plant growth and sinensetin contents are sensitive to many variables, including phytogeographical profiles. This study sought to evaluate the quality of *O. stamineus* obtained from nine locations in Indonesia, predicated on sinensetin levels assessed using TLC-densitometry. Thin Layer Chromatography (TLC) was conducted with silica gel 60 *F*254 as the stationary phase and toluene: ethyl acetate (5:7) and a drop of formic acid for every 10 ml of that solvent mixture as the mobile phase and was analyzed without a derivatization reagent. The created method proved uncomplicated and satisfied the specificity parameters, as indicated by the identical UV spectrum shared between the sinensetin standard and sample (*λ*max = 334 nm). Also, it showed good linearity for sinensetin in the range of 14.5–87 ng/band (*r* = 0.9886). Limits of detection and limits of quantification were 9.03116 and 27.36717 ng/band, respectively. In addition, the method possessed good intra- and interday precision (marked by Relative Standard Deviations (RSDs) of 1.65%–6.47% and 4.97%) and accuracy (95.86, 120.18, and 82.44% recoveries in standard addition with three-level solutions). Of the 14 samples, sinensetin was undetected in two but found in various concentrations in the other 12 samples, from 0.0238 to 0.1533 mg/g. Using a sample from the Tawangmangu area as a reference, three groups of samples were formed: those with lower sinensetin contents (Jakarta Selatan, Lamongan, Jombang, and Sampang), higher sinensetin contents (Surabaya, Mojokerto, Kediri, and Kotabaru), and similar sinensetin contents as the reference sample (Batu, Gresik, and Madiun). The TLC-densitometry designed in this study is straightforward but satisfies the validation parameters; thus, it can be used to qualitatively and quantitatively analyze sinensetin in *O. stamineus*. Overall, *O. stamineus* in different phytogeographical zones in Indonesia has varying levels of sinensetin.

**INTRODUCTION**

*Orthosiphon stamineus* Benth. (Lamiaceae), also known as cat’s whiskers, *kumis kucing* or *misai kucing*, is among the most widely used medicinal herbs in Southeast Asian countries, including Indonesia and Malaysia (Adnyana et al., 2013; Ameer et al., 2012). In Indonesia, the plant is an important ingredient in the products “Jamu Saintifik” and “Fitofarmaka,” recognized for their efficacy in ameliorating hypertension, arthritis, and urinary stones (Indonesia Ministry of Health, 2019, 2022). Many studies have successfully discovered the bioactive compounds associated with these activities (Ashraf et al., 2018; Sarshar et al., 2017; Xu et al., 2020), namely, a variety of derivatives of phenolic acid and flavonoids (Akowuah et al., 2004; Guo et al., 2019). An example is sinensetin, a polymethoxyflavone most responsible for the biological activity of *O. stamineus* not only as an antihypertensive but also as an anticancer, anti-diabetic, antimicrobial, anti-inflamatory, vasorelaxant, and antioxidant agent (Han Jie et al., 2021; Mohamed et al., 2012; Samidurai et al., 2020; Yam et al., 2018; Wang et al., 2022).

*Orthosiphon stamineus* can grow well in different phytogeographical profiles. On the one hand, this characteristic is beneficial because it facilitates the provision of raw materials for traditional medicines for public use and industrial purposes...
in different localities. However, on the other hand, the possibility of variations in material quality can pose a disadvantage to standardized production. It accounts for the fact that the plant’s chemical constituents are sensitive to geographical origins, soil conditions, climate, harvesting process, and postharvest treatments, among others (Bensoussan et al., 2015). Therefore, because phytogeographical configurations affect the compound’s concentration, standardization of *O. stamineus* crude drugs is necessary so as to ensure uniformity of raw materials across various locations.

Herbs can be analyzed for their pharmaceutical quality through many approaches, including markers and compound fingerprints. Several fingerprint-based techniques have been applied to unveil variations in *O. stamineus* grown in different locations, such as combining TLC fingerprinting with chemometrics (Kartini et al., 2020), Fourier Transform-Infra Red (FT-IR) spectroscopy with canonical variate analysis (Rafi et al., 2015), and a virtual chemical sensor based on fast gas chromatography (Sim et al., 2003). Although these fingerprint-based systems are currently developing as they gain more attention from researchers, the conventional technique using chemical markers is still widely used by herbal pharmacopeias in many countries, including the Indonesian Herbal Pharmacopoeia. This is due to the ease of correlation with herbal dosages.

For the above reasons, TLC and High Performance Liquid Chromatography (HPLC) have been proposed to detect and measure sinensetin as a chemical marker and biological activity indicator of *O. stamineus*. This is to address the very few reports on divergent sinensetin contents in the plant samples collected from various phytogeographical zones in Indonesia. Moreover, TLC guarantees speed and simplicity, two criteria highly demanded of routine herbal quality analysis and assessment methods in pharmaceutical industries. In addition, TLC is still broadly used to analyze marker compounds in several compendia, such as the Indonesian Herbal Pharmacopoeia and the Chinese Pharmacopoeia (Shen et al., 2020). Therefore, this study aimed to evaluate the quality of *O. stamineus* obtained from 14 phytogeographical zones in Indonesia based on the concentrations of the marker compound, sinensetin, using TLC-densitometry. Here, a sample from one location, B2P2TOOT Tawangmangu (hereinafter referred to as the Tawangmangu sample), is used as a reference because the area grows *O. stamineus* for the “Jamu Saintifik” products (scientific herbal medicine) nationwide. In addition, B2P2TOOT Tawangmangu is also a service-based herbal clinical testing center to produce “Jamu Saintifik” in Indonesia.

**MATERIALS AND METHODS**

**Materials**

The chemicals used in this study included a sinensetin standard obtained from Sigma-Aldrich (USA), precoated TLC Si gel 60 F254 (20 × 20 cm), and several p.a. grade solvents from Merck KGaA (Darmstadt, Germany).

*Orthosiphon stamineus* leaves were harvested in July–September 2020 from 14 regions in Indonesia. The first eight leaves from the shoots were picked by hand, washed with running water, drained, and then dried by aeration. Afterward, the dried leaves were ground in a blender and sifted with a 45-mesh sieve. Details of the phytogeographical origins of the samples are shown in Table 1, and all samples have been verified by the Center for Information and Development of Traditional Medicines Pusat Informasi dan Pengembangan Obat Tradisional (PIPOT), the University of Surabaya, with Authentication Certificate No. 1434/D.T/I/2021.

**Extract preparation**

Approximately 1 g of *O. stamineus* leaf powder was added with 7 ml of methanol and then extracted using the ultrasound-assisted extraction method for 15 minutes at room temperature. The extract was then separated from the dregs and put into a 10 ml volumetric flask. Extraction was completed by rinsing the dregs with 3 ml of methanol and putting them into the same volumetric flask. The extract volume was then made up to 10.0 ml.

**Table 1.** Phytogeographical details of the *O. stamineus* production areas observed in the research.

| Codes | Regions          | Latitude, longitude       | Elevation (masl) |
|-------|------------------|---------------------------|-----------------|
| 1     | Jakarta Selatan  | 6°15’25” S and 106°46’45” E | 7               |
| 2     | Surabaya         | 7°16’11” S and 112°44’48” E | 7               |
| 3     | Lamongan         | 7°06’25” S and 112°20’08” E | 7.7             |
| 4     | Gresik           | 7°09’58” S and 113°18’07” E | <200            |
| 5     | Batu             | 7°31’52” S and 112°31’12” E | 897             |
| 6     | Ngawi            | 7°33’27” S and 111°17’38” E | 331             |
| 7     | Tawangmangu      | 7°39’50” S and 111°08’04” E | 1,200           |
| 8     | Jombang          | 7°36’52” S and 112°22’10” E | 62              |
| 9     | Sampang          | 7°03’54” S and 113°15’04” E | 63              |
| 10    | Mojokerto        | 7°38’37” S and 112°36’10” E | 650             |
| 11    | Madiun           | 7°39’44” S and 111°36’17” E | 62              |
| 12    | Kediri           | 7°46’07” S and 111°54’36” E | 78              |
| 13    | Badung           | 8°35’53” S and 115°11’52” E | 350             |
| 14    | Kotabaru         | 3°17’32” S and 116°13’05” E | 212             |
Standard solution preparation

Around 1.16 mg of the sinensetin standard was dissolved in sufficient methanol, then transferred to a 10 ml volumetric flask, and added with methanol to 10.0 ml. The mother solution (116 ppm) was then diluted to obtain a working solution with a concentration of 7.25 ppm.

TLC system

The *O. stamineus* leaf extract and sinensetin standard were spotted on a TLC silica gel 60 F254 plate in a 6 mm bandwidth using a CAMAG 100 µl sample syringe (Hamilton, Switzerland). Automatic spotting was conducted with a Linomat 5 TLC Applicator (CAMAG, Switzerland) in a stream of nitrogen gas. The TLC plate was then developed in a twin-through chamber (CAMAG, Switzerland), which had been saturated with the mobile phase (toluene: ethyl acetate = 5:7 and a drop of formic acid for every 10 ml of that solvent mixture) for 30 minutes. An ascending development was performed with an elution distance of 80 mm. The separation results were then documented under 254 and 366 nm UV light without derivatization reagents and then scanned using TLC Scanner 4 (CAMAG, Switzerland) with a 4 × 0.3 mm slit, data resolution of 1 nm/step, and scanning speed of 100 nm/seconds. The densitogram was then analyzed with the winCATS software.

Validation of the analytical method

Specificity study

Eight microliters of the sinensetin standard and 2 and 4 µL of the *O. stamineus* extract samples were applied to the TLC plate and then processed conforming to the TLC system designed in the current research. To ascertain the method’s specificity, the characteristics of the sinensetin in the standard and the samples were cross-compared, including Rf values, UV spectrum profiles, and λ<sub>max</sub> measured with a densitometer. The purity of the samples’ sinensetin band was confirmed by reading the UV spectrum at the beginning, apex, and end of the peak (Spangenberg et al., 2011).

Calculation of linearity, limits of detection (LOD) and quantification (LOQ)

Linearity measures the ability of an analytical procedure to obtain test results, either directly or by mathematical transformations, that correlate linearly with the amount of analyte in the sample within a particular validated range. To determine the procedure’s linearity, a series of the working solutions (2, 4, 6, 8, 10, and 12 µl) were spotted on the plate. After the plate development, the area of the sinensetin band was measured by a densitometer. The purity of the samples’ sinensetin band was confirmed by reading the UV spectrum at the beginning, apex, and end of the peak (Spangenberg et al., 2011).

Evaluation of precision

In the intraday precision testing, 6 µl of the *O. stamineus* extract was spotted repeatedly six times on one plate, while the interday precision spotting was conducted on three different plates on three successive days. Each plate was then analyzed using the TLC-densitometry designed to measure the sinensetin area. Finally, the intraday and interday precision were individually evaluated from the relative standard deviations (%RSD) calculated per plate and from the three plates (Spangenberg et al., 2011).

Evaluation of accuracy

Accuracy represents the proximity between the actual values and the test results of the method being analyzed (theoretical values). Here, accuracy was calculated as a percentage of recoveries using standard addition with multiple solutions. First, multilevel solutions (levels 1–3) were prepared by pipetting three different volumes of the standard sinensetin solutions (i.e., 80, 100, and 120 µl), and each was added with 120, 100, and 80 µl of the sample solutions. Second, to prepare unspiked samples (without the addition of the standard solutions), 120, 100, and 80 µl of the sample solutions were pipetted, and 80, 100, and 120 µl of methanol were added to each. Finally, the multilevel solutions and the unspiked samples were applied to the TLC plate, and this procedure was performed in triplicate. The plates were eluted and then analyzed using a densitometer, and the % recovery was calculated.

Measurement of the sinensetin contents of *O. stamineus*

Amounts of sinensetin in *O. stamineus* grown in 14 different phytogeographical zones in Indonesia were determined using a validated TLC system and calculated as mg/g dry weight (of the leaf).

Data analysis

The sinensetin contents, representing the 14 different phytogeographical profiles, were cross-compared using one-way ANOVA (α = 0.05). Then, a subsequent Tukey test was performed to compare the sinensetin contents of each of the 13 crude drugs against the sample harvested from Tawangmangu (reference sample). The GraphPad Prism Version 5.01 program was used to run these analyses.

RESULTS AND DISCUSSION

Organoletic properties of the crude drugs

The phytogeographical zones explored in the study are relatively diverse in elevation, that is, from 7 to 1,200 masl. However, the crude drugs obtained from the leaves were organoleptically similar, including the brownish-green color and dry and brittle characteristics (Fig. 1).

Specificity

Plant extracts contain different kinds of compounds with various physicochemical characteristics. Two or more different compounds can have similar or even identical polarity, thus appearing as one band on the TLC plate. For example, *O. stamineus* leaves contain several polymethoxy flavonoids, among which the most abundant are 3′-hydroxy-5,6,7,4′-tetramethoxyflavone and sinensetin. These compounds differ in the number and position of the methoxy groups, indicating only slightly different polarity (Hossain and Ismail, 2016).
For these reasons, specificity should be determined to ensure and guarantee that the analytical procedure measures the target compound or that the compound band appearing in the test is the target compound. Specificity was analyzed by comparing the colors and Rf values of the standard and sample bands. In addition, a similarity analysis was also conducted between the UV spectra (200–400 nm) of the standard and the sample to determine specificity.

The TLC chromatograms (Fig. 2) show a band suspected to be sinensetin in the sample bands (tracks b and c). It was a blue fluorescence band under UV light at 366 nm, with a position parallel to the sinensetin standard. Furthermore, the UV spectra of the sinensetin standard and the O. stamineus extract sample (Fig. 3) showed a similar pattern, comprising two peaks, each at maximum wavelengths of 334 and 264 nm. These spectral characteristics are typical of flavonoids in the flavone subclass. In the subsequent analysis, sinensetin in the standard and the extract samples was detected and measured at 334 nm. The selected wavelength corresponds to the one used in a previous study, that is, 338 nm (Arifianti et al., 2014). However, many chose a somewhat different wavelength, 366 nm, for the same purpose (Shehzadi et al., 2018).

Rf values corroborate the similarities shared by the sinensetin standard and samples (Table 2). Densitograms show that both had the same Rf value of 0.31 (Fig. 4). The pure presence of sinensetin in the samples was demonstrated by the values of \( r(s, m) = 0.992192 \) and \( r(m, e) = 0.997264 (>0.99) \) (Patel et al., 2019). It confirms that the suspected band is that of sinensetin and is not mixed with other compounds. From these findings, it can be inferred that the TLC method developed in this study has good specificity or is specific to detecting sinensetin levels in O. stamineus.

Linearity, the limits of detection and quantification

To determine linearity, the correlation coefficient \( (r) \) obtained from the standard curve was observed. The curve was formed using triplicate measurements \( (n = 3) \), that is, spotting different volumes of the sinensetin standard (7.25 ppm): 2, 4, 6, 8, 10, and 12 \( \mu \)l or equivalent to 14.5–87 ng/band. Based on the TLC-derived chromatogram and 2D densitogram of the standard sinensetin solution (Fig. 5), the linear regression model showed a positive linear correlation between the mass of sinensetin and the fluorescence intensity of the compound band and the peak area (Fig. 6). The \( r \)-value obtained from the model was 0.98858, meaning that the linear relationship holds for 14.5 to 87 ng/band of sinensetin for each area (Hashim et al., 2016).

LOD and LOQ, calculated from the standard curve \( (n = 3) \), were 9.03116 ng/band and 27.36717 ng/band. These values were about two times smaller than those identified in a previous study that used High Performance Thin Layer Chromatography (HPTLC) to analyze sinensetin and three other compounds in the O. stamineus leaf extract simultaneously, that is, 17.26 and 52.3 ng/spot (Hashim et al., 2016). LOD (and LOQ) indicates the smallest amount of analyte detectable (and quantifiable) by the analytical procedure used with reasonable statistical certainty. HPTLC is different from TLC as it uses a smaller particle size for the stationary phase and, consequently, produces better analytical performance, but HPTLC plates are more pricey than TLC plates (Srivastava, 2010; Zlatkis and Kaiser, 2011). The study results suggested that the developed TLC is sensitive and, thus, sufficient for evaluating sinensetin contents in O. stamineus leaves.

Precision

Precision describes the closeness of agreement between multiple sample replications and the random error in an analytical procedure. Figure 7 shows one of the chromatograms of the six samples spotted on a TLC plate used in the intraday and interday
precision testing. Table 3 provides the amounts of sinensetin read from it.

Table 3 indicates that the TLC-densitometry designed for a single sinensetin assay for *O. stamineus* has good intraday and interday precision, as shown by RSDs of 1.65%–6.47% and 4.97%, respectively. With a different precision test design, the HPTLC-densitometer in a previous study has been found to also have good intraday and interday precision for sinensetin, with RSDs of 3.76%–4.38% and 3.48%–4.24% (Shehzadi *et al.*, 2018).
Accuracy was analyzed using standard addition with multiple solutions (three levels) and unspiked samples (six spots for the TLC). The three levels produced recoveries of 95.86%, 120.18%, and 82.44% (Table 4), which correspond to previous studies that used an HPTLC-densitometer (Akowuah et al., 2006; Shehzadi et al., 2018). Because the recoveries were in the range of 80%–120%, the proposed method is therefore accurate (Riyanto, 2014).

Sinensetin levels in *O. stamineus* from various phytogeographical zones

To determine the sinensetin concentrations, each *O. stamineus* extract sample was spotted with the appropriate volume on a TLC plate and analyzed using the designed and validated procedure. TLC-derived chromatograms of the 14 samples and their sinensetin measurement results are shown in Figure 8 and Table 5. Table 5 shows that samples from the Ngawi and Badung areas contained minute sinensetin (below the LOQ). Compared with the other samples, their other metabolites were also extremely low (Fig. 8). On the contrary, the other 12 samples showed varying levels of sinensetin, ranging from 0.0238 to 0.1533 mg/g. One-way ANOVA results revealed a significant difference in the sinensetin levels of the 14 *O. stamineus* samples ($p < 0.0001$). Because this study aimed to determine the quality profile of the *O. stamineus* leaves obtained from various locations with different phytogeographic characteristics, a post hoc Tukey test was performed to statistically compare each sample with the reference sample (from the Tawangmangu area). Based on the analysis results (Fig. 9), the samples can be clustered into three groups. The group containing significantly lower sinensetin than the reference sample was comprised of the Jakarta Selatan, Lamongan, Jombang, and Sampang samples. On the contrary, the one with significantly higher sinensetin concentrations consisted of the Surabaya, Mojokerto, Kediri, and Kotabaru samples. The last group considered the Batu, Gresik, and Madiun samples as...
Table 3. Sinensetin areas ($\lambda = 334$ nm) of six $O. stamineus$ samples measured for the intraday and interday precision testing.

| Replications | Day 1     | Day 2     | Day 3     |
|--------------|-----------|-----------|-----------|
| 1            | 2,960.70  | 2,490.27  | 2,628.12  |
| 2            | 2,991.81  | 2,526.84  | 2,718.25  |
| 3            | 2,928.37  | 2,577.36  | 2,754.36  |
| 4            | 2,880.37  | 2,687.37  | 2,689.59  |
| 5            | 2,855.15  | 2,856.10  | 2,738.82  |
| 6            | 2,937.83  | 2,901.24  | 2,699.74  |
| Mean ± SD    | 2,925.71 ± 50.56 | 2,673.20 ± 173.05 | 2,704.81 ± 44.57 |
| RSD (%)      | 1.73      | 6.47      | 1.65      |

Intraday precision (%RSD. $n = 6$) = 1.65–6.47

Interday precision (%RSD. $n = 3$) = 4.97

Table 4. Accuracy test results of the proposed TLC method for sinensetin measurements in $O. stamineus$.

| Levels | Tracks | Areas | Total sinensetin (ng) | Measured sinensetin (ng) | Theoretical sinensetin (ng) | Recovery (%) |
|--------|--------|-------|-----------------------|--------------------------|-----------------------------|--------------|
| 1      | Unspiked samples | 1,538.20 ± 151.51 | 15.14 ± 3.04 | 16.68 ± 0.31 | 17.4 | 95.86 ± 1.75 |
|        | Standard addition | 2,369.13 ± 15.21 | 31.82 ± 0.31 | 26.14 ± 0.28 | 21.75 | 120.18 ± 1.29 |
| 2      | Unspiked samples | 1,146.47 ± 82.80 | 7.28 ± 1.66 | 13.10 ± 5.32 | 21.50 | 82.44 ± 8.81 |
|        | Standard addition | 2,448.87 ± 13.99 | 33.42 ± 0.28 | 21.52 ± 2.30 | 26.1 | 82.44 ± 8.81 |
| 3      | Unspiked samples | 1,436.97 ± 264.85 | 13.10 ± 5.32 | 21.52 ± 2.30 | 26.1 | 82.44 ± 8.81 |
|        | Standard addition | 2,508.91 ± 114.6 | 34.62 ± 2.30 | 21.52 ± 2.30 | 26.1 | 82.44 ± 8.81 |

*Mean ± SD ($n = 3$)

Figure 8. TLC-derived chromatograms of the sinensetin standard (std) and $O. stamineus$ samples from 14 phytogeographically diverse sites (1–14) read under 254 nm (A) and 366 nm (B) UV light.

Table 5. Sinensetin levels of the $O. stamineus$ samples harvested from 14 phytogeographical zones in Indonesia.

| Sample codes | Origins of sample | Sinensetin contents (mg/g) |
|--------------|-------------------|---------------------------|
| 1            | Jakarta Selatan   | 0.0238 ± 0.0013           |
| 2            | Surabaya          | 0.0556 ± 0.0102           |
| 3            | Lamongan          | 0.0250 ± 0.0043           |
| 4            | Gresik            | 0.0485 ± 0.0045           |
| 5            | Batu              | 0.0458 ± 0.0031           |
| 6            | Ngawi             | *                         |
| 7            | Tawangmangu       | 0.0394 ± 0.0073           |
| 8            | Jombang           | 0.0266 ± 0.0045           |
| 9            | Sampang           | 0.0255 ± 0.0033           |
| 10           | Mojokerto         | 0.0564 ± 0.0086           |
| 11           | Madiun            | 0.0333 ± 0.0058           |
| 12           | Kediri            | 0.1533 ± 0.0097           |
| 13           | Badung            | *                         |
| 14           | Kotabaru          | 0.0523 ± 0.0073           |

*: not measurable (sinensetin level < LOQ), data were obtained from the mean ± SD of triplicate measurements ($n = 3$).
having no significantly different sinensetin contents from the reference sample.

The study results proved that the amounts of sinensetin found in *O. stamineus* are influenced by the plant’s phytogeographical origins. However, further research is required to determine whether or not the location’s elevation and other contributing variables account for such differences. The findings of this study strengthen the data of previous studies which stated that there was a diversity of agronomic characters (accumulated height gain for 8 weeks after planting; number of secondary branches and secondary branch internodes; length, width, and leaf area index; and average dry weight of stems and leaves per 4.41 m²) and the sinensetin content of *O. stamineus* from *ex situ* collections from Jawa Barat, Jawa Tengah, and Jawa Timur (Febjislami et al., 2018). These findings provide scientific evidence that justifies the essence of factoring in geographical conditions in cultivating *O. stamineus* to obtain standardized harvests with consistent sinensetin contents.

**CONCLUSION**

The TLC-densitometry designed in the study is straightforward but satisfies the validation parameters; thus, it can be used to qualitatively and quantitatively analyze sinensetin in *O. stamineus*. In addition, the study discovered that the sinensetin contents of the extracts prepared from *O. stamineus* vary across the plant’s phytogeographical zones in Indonesia. For future research, it is recommended to utilize the method for other phytogeographical locations in Indonesia and other countries.

**AUTHORS’ CONTRIBUTIONS**

Kartini Kartini conceptualized the study; Kartini Kartini and Rizky Eka Putri conducted the experiment; Kartini Kartini, Rizky Eka Putri, and Ryanto Budiono analyzed the results. All authors reviewed the manuscript.

**FINANCIAL SUPPORT**

This research was funded by the Indonesian Ministry of Education, Culture, Research and Technology with the contract number: 063 /SP-Lit/LPPM-01/KemendikbudRistek/Multi/FF/III/2022.

**CONFLICTS OF INTEREST**

The authors report no financial or any other conflicts of interest in this work.

**ETHICAL APPROVALS**

This study does not involve experiments on animals or human subjects.

**DATA AVAILABILITY**

All data generated and analyzed are included in this research article.

**PUBLISHER’S NOTE**

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

**REFERENCES**

Adnyana IK, Setiawan F, Insanu M. From ethnopharmacology to clinical study of *Orthosiphon stamineus* Benth. studies, 2013; 1(2): 66-73.

Akowuah G, Zhari I, Norhayati I, Sadikun A, Khamshah S. Sinensetin, eupatorin, 3′-hydroxy-5, 6, 7, 4′-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. Food Chem, 2004; 87(4):559–66.

Akowuah G, Zhari I, Sadikun A, Norhayati I. HPTLC densitometric analysis of *Orthosiphon stamineus*. leaf extracts and inhibitory effect on xanthine oxidase activity. Pharm Biol, 2006; 44(1):65–70.

Ameer OZ, Salman IM, Asmawi MZ, Ibraheem ZO, Yam MF. *Orthosiphon stamineus*: traditional uses, phytochemistry, pharmacology, and toxicology. J Med Food, 2012; 15(8):678–90.

Arifianti L, Oktarina RD, Kusumawati I. Pengaruh jenis pelarut pengekstraksi terhadap kadar sinensetin dalam ekstrak daun *Orthosiphon stamineus* Benth. E-J Planta Husada, 2014; 2(1):1–4.

Ashraf K, Sultan S, Adam A. *Orthosiphon stamineus* Benth. is an outstanding food medicine: review of phytochemical and pharmacological activities. J Pharm Bioall Sci, 2018; 10(3):109.

Bensousan A, Lee S, Murray C, Bourcher S, Van Der Kooy F, Pearson JL, Liu J, Chang D, Kho O. Choosing chemical markers for quality assurance of complex herbal medicines: Development and application of the herb MaRS criteria. Clinical Pharmacology & Therapeutics, 2015; 97(6): 628-640.

Febjislami S, Melati M, Kurniawati A, Wahyu Y. Karakter agronomi dan kadar sinensetin beberapa akses tanaman kumis kucing (*Orthosiphon stamineus*). Jurnal Hortikultura Indonesia, 2018; 9(3):206–15.
Guo Z, Liang X, Xie Y. Qualitative and quantitative analysis on the chemical constituents in Orthosiphon stamineus Benth. using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal, 2019; 164:135–47.

Han Jie L, Jantan I, Yusoff SD, Jalil J, Husain K. Sinensetin: an insight on its pharmacological activities, mechanisms of action and toxicity. Front Pharmacol, 2021; 11:533404.

Hashim S, Beh HK, Hamil MSR, Ismail Z, Majid AMSA. High-performance thin-layer chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of Orthosiphon stamineus. Pharmacogn Res, 2016; 8(4):238.

Hossain MA, Ismail Z. Quantification and enrichment of sinensetin in the leaves of Orthosiphon stamineus Benth. from different origins. Pharmacogn J, 2020; 12(1):1683–91.

Indonesia Ministry of Health. Eleven scientific herbal medicines “Jamu Saintifik”, Karanganyar, center for research and development of medicinal plants and traditional medicines (in Indonesia). Indonesia Ministry of Health, Jakarta, Indonesia, 2019.

Indonesia Ministry of Health. “Fitofarmaka” Formulary. Ministry of Health (in Indonesian). Indonesia Ministry of Health Jakarta, Indonesia, 2022.

Kartini K, Jayani NIE, Hadiyat MA, Avanti C. Thin layer chromatography fingerprinting and clustering of Orthosiphon aristatus Benth. from different origins. Pharmacogn J, 2020; 12(1):1683–91.

Mohamed EAH, Siddiqui MJA, Ang LF, Sadikun A, Chan SH, Tan SC, Asmawi MZ, Yam MF. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from Orthosiphon stamineus Benth as anti-diabetic mechanism. BMC Complement Altern Med, 2012; 12(1):1–7.

Patel LJ, Raval MA, Patel SG, Patel AJ. Development and validation of stability indicating high-performance thin-layer chromatographic (HPTLC) method for quantification of asiaticoside from Centella asiatica L. and its marketed formulation. J AOAC Int, 2019; 102(4):1014–20.

Rafi M, Purwakusumah ED, Ridwan T, Barus B, Sutandi A, Darusman LK. Geographical classification of java tea (Orthosiphon stamineus) from java Island by FTIR spectroscopy combined with canonical variate analysis. Jurnal Sains dan Matematika Universitas Diponegoro, 2015; 23:25–31.

Riyanto R. Validasi & verifikasi metode Uji Deepublish. Deepublish, Yogyakarta, Indonesia.

Srivastava M. High-performance thin-layer chromatography (HPTLC). Springer Science & Business Media, Berlin, Germany, 2010.

Wang Q, Wang J, Li N, Liu J, Zhou J, Zhang P, Chen H. A systematic review of Orthosiphon stamineus Benth. in the treatment of diabetes and its complications. Molecules, 2022; 27(2):444.

Yam MF, Tan CS, Shibao R. Vasorelaxant effect of sinensetin via the NO/sGC/cGMP pathway and potassium and calcium channels. Hypertens Res, 2018; 41(10):787–97.

Zlatkis A, Kaiser REHPTLC-high performance thin-layer chromatography. Elsevier, Amsterdam, Netherlands, 2011.

Shehzadi N, Zahid F, Naheed S, Javed R, Qamar S, Sher R, Irfan Bukhari N, Ismail Z, Sadikun A. Quantification of sinensetin in extracts of Orthosiphon stamineus using high performance thin-layer chromatography. Pakistan Journal of Pharmacy, 2018; 29(1):12-19.

Shen M-R, Ye Y, Shi S-M. Development of chromatographic technologies for the quality control of Traditional Chinese Medicines in the Chinese Pharmacopoeia. J Pharm Anal., 2020; 11(2):155-62.

Sim CO, Ahmad MN, Ismail Z, Othman AR, Noor NaM, Zaihidee EM. Chemometric classification of herb–Orthosiphon stamineus according to its geographical origin using virtual chemical sensor based upon fast GC. Sensors, 2003; 3(10):458–71.

How to cite this article:
Kartini K, Putri RE, Budiono R. Quantification of sinensetin in Orthosiphon stamineus from various phytogeographical zones in Indonesia. J Appl Pharm Sci, 2023; 13(03):183-191.