

Integration of TLC-, FTIR-, and ¹H-NMR-based metabolomics and chemometric analyses for discriminating the geographical origin of *Orthosiphon aristatus* (Blume) Miq.

Fitriana Hayyu ARIFAH^{*,**}, Abdul ROHMAN^{***}, Wawan SUJARWO^{****},
Agung Endro NUGROHO^{*****}

Integration of TLC-, FTIR-, and ¹H-NMR-based metabolomics and chemometric analyses for discriminating the geographical origin of *Orthosiphon aristatus* (Blume) Miq.

SUMMARY

Orthosiphon aristatus (Blume) Miq. is a medicinal plant with multiple pharmacological effects. *O. aristatus* from different geographical origins have distinct phytochemical compound profiles. This study aims to provide a targeted thin-layer chromatography (TLC)-based metabolomics approach for determining sinensetin levels and to use metabolomics approaches and chemometric analyses based on untargeted Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (¹H-NMR) to discriminate *O. aristatus* from three geographical locations in Menoreh Hill, Indonesia. TLC was used to analyze sinensetin levels from three different geographical origins. FTIR and ¹H-NMR were respectively employed to analyze different functional groups and ¹H-NMR spectra profiles and to discriminate between different geographical origins using chemometric analyses (i.e., unsupervised principal component analysis [PCA] and cluster analysis [CA]). TLC can determine the sinensetin levels and phytochemical profiles of *O. aristatus* from different geographical origins. Meanwhile, the FTIR spectra indicated several phytochemical classes, including alkane, alkene, aldehyde, alcohol/phenol, and aromatics. The ¹H-NMR spectra showed several metabolite classes, including fatty acids, amino acids, carbohydrates, phenolics, flavonoids, and terpenoids. TLC, FTIR, and ¹H-NMR, combined with chemometrics, can be applied to metabolomics analyses of *O. aristatus* from different geographical origins.

Keywords: *Orthosiphon aristatus* (Blume) Miq., phytochemistry, metabolomics, chemometrics, geographical origin.

***Orthosiphon aristatus* (Blume) Miq.'nin Coğrafi Kökeninin Ayırt Edilmesine Yönelik TLC-, FTIR- ve ¹H-NMR Temelli Metabolomik ve Kemometrik Analizlerin Entegrasyonu**

ÖZ

Orthosiphon aristatus (Blume) Miq., çok sayıda farmakolojik etkiye sahip tıbbi bir bitkidir. Farklı coğrafi kökenlerden elde edilen *O. aristatus* örnekleri, farklı fitokimyasal bileşik profilleri göstermektedir. Bu çalışmanın amacı, Endonezya'daki Menoreh Tepesi bölgesinde bulunan üç farklı coğrafi konumdan elde edilen *O. aristatus* örneklerini ayırt etmek için sinensetin düzeylerinin belirlenmesine yönelik hedefli ince tabaka kromatografisi (İTK) temelli bir metabolomik yaklaşım ile hedeflenmemiş Fourier dönüşümlü kızılötesi (FTIR) spektroskopisi ve proton nükleer manyetik rezonans (¹H-NMR) temelli metabolomik yaklaşımlar ve kemometrik analizler sunmaktır. Üç farklı coğrafi kökenden elde edilen örneklerde sinensetin düzeylerinin analizi için İTK kullanılmıştır. FTIR ve ¹H-NMR yöntemleri sırasıyla farklı fonksiyonel grupların ve ¹H-NMR spektral profillerinin analizinde kullanılmış; farklı coğrafi kökenlerin ayırt edilmesi için kemometrik analizler (yani, denetimsiz temel bileşen analizi [PCA] ve kümeleme analizi [CA]) uygulanmıştır. İTK, farklı coğrafi kökenlerden elde edilen *O. aristatus* örneklerinde sinensetin düzeylerini ve fitokimyasal profilleri belirleyebilmiştir. FTIR spektrumları, alkanlar, alkenler, aldehitler, alkol/fenoller ve aromatik bileşikler dâhil olmak üzere çeşitli fitokimyasal sınıfların varlığını göstermiştir. ¹H-NMR spektrumları ise yağ asitleri, amino asitler, karbonhidratlar, fenolikler, flavonoidler ve terpenoidler gibi çeşitli metabolit sınıflarını ortaya koymuştur. İTK, FTIR ve ¹H-NMR teknikleri, kemometrik yöntemlerle birlikte kullanıldığında, farklı coğrafi kökenlerden elde edilen *O. aristatus* örneklerinin metabolomik analizlerinde etkili bir şekilde uygulanabilir.

Anahtar Kelimeler: *Orthosiphon aristatus* (Blume) Miq., fitokimya, metabolomik, kemometri, coğrafi köken.

Received: 06.01.2026
Revised: 26.03.2026
Accepted: 01.06.2026

* ORCID: 0000-0002-4294-1146, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

** ORCID: 0000-0002-4294-1146, Research Group of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

*** ORCID: 0000-0002-1141-7093, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

**** ORCID: 0000-0001-8858-9769, Ethnobiology Research Group, Research Center for Biology, National Research and Innovation Agency (BRIN), Bogor, Indonesia

***** ORCID: 0000-0001-7840-8493, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

° Corresponding Author; Agung Endro Nugroho
E-mail: nugroho_ac@ugm.ac.id

INTRODUCTION

Plants contain two metabolites with different functions: primary and secondary metabolites (Mais et al., 2018). Primary metabolites, known as general metabolites, are limited in plants and perform basic functions for cell survival (Adhikary & Dasgupta, 2023; Çiçek et al., 2024). In contrast, secondary metabolites contribute to ecologically important processes, including signaling, resistance, and competition, which are also relevant to plant breeding (Çiçek et al., 2024). They are also essential in traditional medicines, folk uses, pharmaceuticals, fragrances, flavorings, and natural sweeteners (Adhikary & Dasgupta, 2023). These metabolites are classified into different classes and subclasses: terpenes, phenolics, flavonoids, alkaloids, and others (Adhikary & Dasgupta, 2023). Therefore, measuring both the qualitative and quantitative characteristics of these metabolites is crucial (Mais et al., 2018). This measurement is termed metabolomics, which has been used as a powerful tool for phytochemical analyses (Mais et al., 2018; Shah et al., 2023).

Metabolomics involves examining small molecules (<1500 Da), which are useful for the identification and quantification of all metabolites in biological systems (Ellis & Goodacre, 2012; Markley et al., 2017; Onuh & Aluko, 2019). Metabolomics is divided into two approaches: untargeted and targeted (Cuadros-Rodríguez et al., 2016). Untargeted metabolomics can be applied to determine all metabolites detected in a particular analysis without identification and quantification and thus does not provide specific metabolite information (Cuadros-Rodríguez et al., 2016; Shah et al., 2023). Meanwhile, targeted metabolomics focuses on specific metabolites and enables the profiling of hundreds of known metabolites in a single analysis (McMillen et al., 2023). Several instruments can be used for untargeted metabolomics (e.g., Fourier transform infrared [FTIR] spectroscopy, nuclear magnetic resonance [NMR], mass spectrometry [MS], and UV-visible) and targeted metabolomics

(e.g., high-performance liquid chromatography, gas chromatography, and thin-layer chromatography [TLC]) (Arifah et al., 2026; Kharbach et al., 2020).

Orthosiphon aristatus (Blume) Miq. is a medicinal plant of the Lamiaceae family (United States Department of Agriculture, 2023). It is widely used as an antidiabetic remedy in local communities in both single- and mixed formulations (Arifah et al., 2022). The ancient Indonesian book *Cabe Puyang Warisan Nenek Moyang* features four antidiabetic formulas from *O. aristatus* (Mardiswojo & Rajakmangunsudarso, 1987), while several provinces prescribe single antidiabetic formulations (Arifah et al., 2022). Phytochemical studies have identified antidiabetic compounds in *O. aristatus*, such as pimarane diterpenes from aerial parts (siphonol B and D and orthosiphonol F, G, I, and N) and flavonoids from leaves (sinensetin) (Mohamed et al., 2012). The antidiabetic mechanisms of *O. aristatus* have been explored, including the stimulation of insulin secretion (Sriplang et al., 2007), inhibition of glucose absorption in the intestine (Maulana et al., 2022), promotion of glucose uptake (Indariani et al., 2014), regulation of hepatic glycogen (Rao et al., 2014), increase in insulin expression via PI3K and Akt (Lee et al., 2015), insulin-mimicking effects (Azhari et al., 2015), and regulation of insulin signaling through PTP-1B inhibition (Nguyen et al., 2019).

Phytochemical compounds are influenced by geographical cultivation, storage times, harvest times, and processing methods (Mais et al., 2018). However, although numerous studies have investigated the phytochemistry of *O. aristatus*, only a few have applied comprehensive analytical methods as evaluation tools, such as TLC, FTIR, and proton NMR (¹H-NMR) combined with chemometric analysis. Several studies of the influence of treatments on the phytochemical studies of *O. aristatus* using chemical tools have been reported. Adulteration of *O. aristatus* and its adulterations have been analyzed using a combination of UHPLC-Q-TOF-MS with a feature-based

molecular network (Yong et al., 2025). Thus, a study of quality control of the various *O. aristatus* leaf extracts using HPLC has been reported (Ahda et al., 2025). A study from China reported on the analysis of chemical compounds and their metabolites in *O. aristatus* using UHPLC-Q-extractive orbitrap-HRMS and AF-ADESI-MSI techniques (Ouyang et al., 2024). To the best of our knowledge, there has been no comprehensive analysis of *O. aristatus* from Menoreh Hill, Indonesia. Thus, this study aims to discriminate among *O. aristatus* samples from three geographical locations in Menoreh Hill, Indonesia, using TLC, FTIR, and ¹H-NMR combined with chemometric analysis.

MATERIALS AND METHODS

Materials and chemicals

Sinensetin (Sigma-Aldrich, Germany), 70% ethanol (General Labora, Indonesia), silica gel 60 F₂₅₄ plate (Merck, Germany), deuterated methanol (CD₃OD), trimethylsilylpropanoic acid (TMSP), and all solvents were of analytical grade (Merck, Germany).

Plant collection and preparation

O. aristatus was collected from three areas: (1) Suroloyo Peak, Samigaluh, Kulon Progo, Special Region of Yogyakarta, Indonesia (high altitude); (2) Sidoharjo Village, Samigaluh, Kulon Progo, Special Region of Yogyakarta, Indonesia (medium altitude); and (3) Hargorojo Village, Bagelen, Purworejo, Central Java, Indonesia (low altitude). The collection was conducted in November 2022 and December 2022. These sites represented different altitudes in Menoreh Hill. The plant materials used in this study were aerial parts (herbs), defined as those portions above the ground (Kementerian Kesehatan Republik Indonesia, 2017). The plant was identified by Dr. Djoko Santosa at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia, under the registration number 17.15.11/UN1/FFA.2/S1/PT/2022. *O. aristatus* herbs were cut into small pieces (about 3 cm long), cleaned with tap water, and dried in an oven at 50°C for 2 days. The dried herbs were ground and passed through an 18-mesh sieve.

Thin-layer chromatography

Extraction and characterization

O. aristatus powder (10 g) was extracted using 100 mL of 70% v/v ethanol (1:10, w/v) for 24 h, with occasional stirring in the first 6 h (Department of Health of the Republic of Indonesia, 2008). The mixture was then filtered, and the filtrate was evaporated using a water bath at 50°C to obtain thick extracts. The ethanolic extract of *O. aristatus* was characterized based on yield, organoleptic properties, and phytochemical composition.

Condition optimization

The mobile phase and maximum wavelength of the TLC system were optimized before quantifying the sinensetin level. Six mobile phases were tested: (1) chloroform–ethyl acetate (6:4 v/v) (Akowuah et al., 2006), (2) ethyl acetate–chloroform (7.5:2.5 v/v) (Mohamed et al., 2011), (3) toluene–ethyl acetate–formic acid (3:7:0.1 v/v/v) (Hashim et al., 2016), (4) chloroform–dichloromethane–ethyl acetate (7:4:1 v/v/v) (Kartini et al., 2020), (5) *n*-hexane–ethyl acetate (3:7 v/v) (Faramayuda et al., 2021), and (6) toluene–ethyl acetate (5:7 v/v), with a drop of formic acid for each 10 mL of the mobile phases (Kartini et al., 2022). The TLC system scanned the maximum wavelength of the sinensetin compound in the range of 200–600 nm.

Quantification of the sinensetin level

Standard solutions of sinensetin were prepared by dissolving it in methanol pro analysis (p.a.) to obtain a 1000-μg/mL stock solution. The stock solution was subsequently diluted into a series of concentrations (0.625, 1.25, 2.5, 5.0, 7.5, and 10.0 μg/mL). The ethanolic extract of *O. aristatus* was prepared by dissolving 100 mg of extract in methanol p.a. and diluting to 10 mL in a volumetric flask. The application volume was 5 μL. The sinensetin level was quantified using TLC on silica gel 60 F₂₅₄ with chloroform–ethyl acetate (6:4 v/v) as the mobile phase at λ_{max} of 335 nm (Akowuah et al., 2006).

Data analysis

The analysis of the TLC profile of *O. aristatus* was divided into two approaches: (1) qualitative analysis of TLC spots and (2) quantification of the sinensetin level. The data were presented as mean $\pm$ standard error of the mean and analyzed using SPSS version 23 at a significance level of $p < 0.05$. Group comparisons were conducted using the analysis of variance test, followed by a post-hoc Tukey test for normal and homogeneous data (Hamadi et al., 2012). Abnormal and/or nonhomogeneous data were analyzed using the Mann–Whitney test (Arifah et al., 2025).

FTIR–ATR spectroscopy

FTIR measurement

O. aristatus powder was placed on the attenuated total reflectance (ATR) diamond crystals of a Nicolet iS10 spectrophotometer coupled with a deuterated triglycine sulfate detector. Background reading was conducted before the samples were placed onto the ATR diamond to avoid friction between the diamond and any spectrum in the air (Fatmarahmi et al., 2022). The samples were measured in the wavenumber region of $4000\text{--}650\text{ cm}^{-1}$ with a resolution of 8 cm^{-1} at 32 scans per minute. The data were visualized in the form of absorbance values, and measurements were performed in triplicate.

Data analysis

The analysis of the infrared (IR) spectra of *O. aristatus* powder was divided into two approaches: (1) qualitative analysis of functional groups and (2) chemometric analysis of selected wavenumbers. The IR spectra were interpreted by comparing spectra (functional groups) in this study and in previous works. Chemometric analysis was conducted using MetaboAnalyst version 5.0. Unsupervised principal component analysis (PCA) and cluster analysis (CA) were conducted. The PCA model was divided into two visualizations: a score plot and a biplot. Meanwhile, the CA model was divided into two visualizations: a dendrogram and a heatmap.

NMR spectroscopy

NMR measurement

One hundred milligrams of *O. aristatus* powder was added to 1 mL of CD_3OD (which contained the internal standard TMSP [0.001%]), vortexed for 1 min, sonicated for 30 min, and centrifuged at 5000 rpm for 2 min. TMSP was used as an internal standard to calibrate chemical shifts on ^1H -NMR signals at 0 ppm (Pariyani et al., 2017; Tajidin et al., 2019). The clear deuterated supernatant was put into an NMR tube for ^1H -NMR measurement in a JNM-ECZ500R 500-Hz NMR spectrometer. The proton measurement was conducted with a suppressed H_2O signal at a chemical shift of 4.86 ppm.

Data analysis

The ^1H -NMR spectra of *O. aristatus* were processed through phase correction, baseline correction, and binning and analyzed through spectral interpretation and chemometric analysis. ^1H -NMR spectra interpretation was conducted by comparing the spectra (chemical shift) in this study and in previous works. The spectra were binned in the region of $\delta\text{ }0.00\text{--}10.00\text{ ppm}$ by an equal width ($\delta\text{ }0.04$). The signals in the region of $\delta\text{ }4.70\text{--}4.96$ (water residual) and $\delta\text{ }3.28\text{--}3.33$ (methanol residual) were excluded for statistical analysis. The processed data were then subsequently analyzed (Tajidin et al., 2019) in MetaboAnalyst version 5.0 using PCA and CA. The PCA model was divided into two visualizations: a score plot and a biplot. Meanwhile, the CA model was divided into two visualizations: a dendrogram and a heatmap.

RESULTS AND DISCUSSION

Sample identification, extraction, and TLC profiles

Samples of *O. aristatus* are presented in Figure 1. The ethanolic extracts of *O. aristatus* appeared as a dark green, bitter, odorous, and consistent paste. The yields of these extracts from Suroloyo, Samigaluh, and Bagelen were $9.78\% \pm 0.09\%$ w/w, $12.86\% \pm 0.18\%$ w/w, and $10.09\% \pm 0.05\%$ w/w, respectively.



Figure 1. *Orthosiphon aristatus* samples from three locations
(a) Suroloyo, (b) Samigaluh, and (c) Bagelen

Optimization of the TLC system

TLC systems (mobile phase and maximum wavelength) were optimized before the sinensetin levels were quantified in the ethanolic extract of *O. aristatus*. Mobile phase optimization was used to obtain mobile phase compositions that can separate sinensetin from the extract with an R_f value of 20–80, resulting in

many spots in the plate and a wider distance between them (Prajapati et al., 2022). Six mobile phase compositions were tested (Figure 2.), giving R_f values of 65, 60, 59, 28, 40, and 38, respectively. Among these, chloroform–ethyl acetate (6:4 v/v) was selected, yielding an R_f value of 65 and the most spots. Scanning the maximum wavelength revealed that sinensetin had a maximum wavelength of 335 nm.

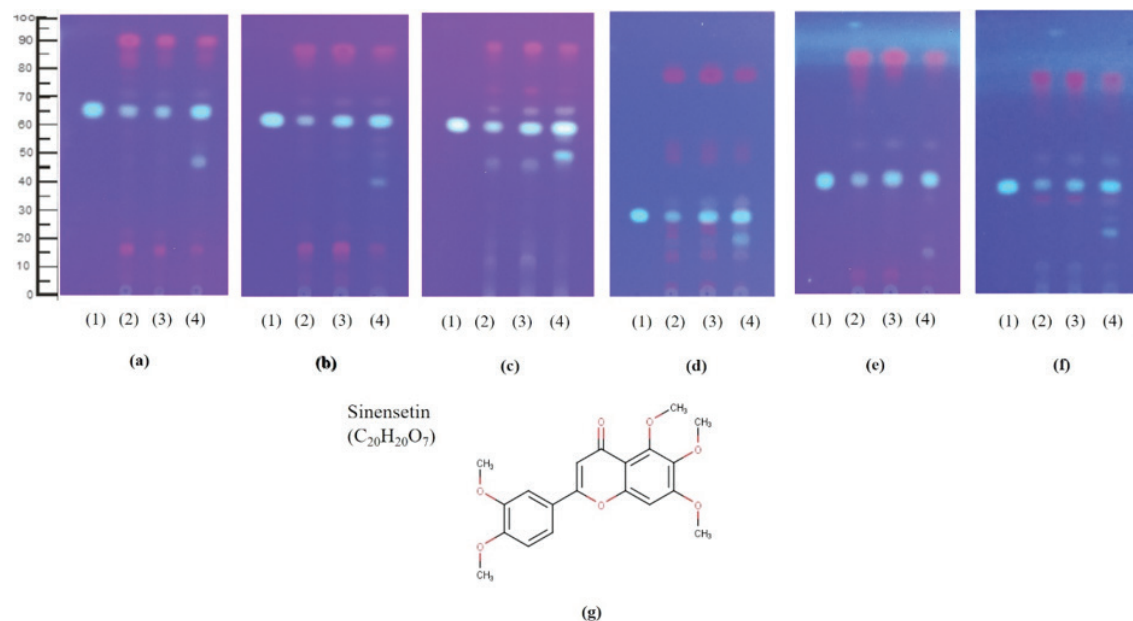


Figure 2. TLC profile of sinensetin in several mobile phase variations. (1) Sinensetin, (2) ethanolic extract from Suroloyo, (3) ethanolic extract from Samigaluh, (4) ethanolic extract from Bagelen. (a) Chloroform–ethyl acetate (6:4 v/v)^[1], (b) ethyl acetate–chloroform (7.5:2.5 v/v)^[2], (c) toluene–ethyl acetate–formic acid (3:7:0.1 v/v/v)^[3], (d) chloroform–dichloromethane–ethyl acetate (7:4:1 v/v/v)^[4], (e) *n*-hexane–ethyl acetate (3:7 v/v)^[5], (f) toluene–ethyl acetate (5:7 v/v) and a drop of formic acid for each 10 mL of the mobile phase^[6], and (g) sinensetin structure. [1] Akowuah et al., 2006; [2] Mohamed et al., 2011; [3] Hashim et al., 2016; [4]

Kartini et al., 2020; [5] Faramayuda et al., 2021; [6] Kartini et al., 2022

TLC profile and sinensetin level

The TLC profile for sinensetin quantification in the ethanolic extract of *O. aristatus* under UV₃₆₆ from Suroloyo, Samigaluh, and Bagelen is presented in Figure 3. The calibration curve equation of sinensetin was $Y = 155.89x + 651.1$ ng, with a correlation coefficient (R) of 0.998. The sinensetin levels of the ethanolic extract of *O. aristatus* from Suroloyo, Samigaluh, and Bagelen were $0.044\% \pm 0.000\%$ w/w, $0.075\% \pm 0.002\%$ w/w, and $0.109\% \pm 0.001\%$ w/w, respectively. The TLC profile of the ethanolic extract of

O. aristatus from Suroloyo under UV₃₆₆ showed seven spots at hRf values of 2 (red), 4 (red), 7 (red), 55 (blue/sinensetin), 61–62 (blue), 79 (red), and 85–86 (red). The TLC profile of the ethanolic extract of *O. aristatus* from Samigaluh under UV₃₆₆ showed seven spots at hRf values of 2 (red), 4 (red), 7 (red), 53–54 (blue/sinensetin), 60 (blue), 78–79 (red), and 84–85 (red). Thus, the TLC profile of the ethanolic extract of *O. aristatus* from Bagelen under UV₃₆₆ showed eight spots at hRf values of 4 (red), 6 (red), 35–37 (blue), 37–40 (blue), 53–56 (blue/sinensetin), 60–63 (blue), 85 (red), and 89 (red).

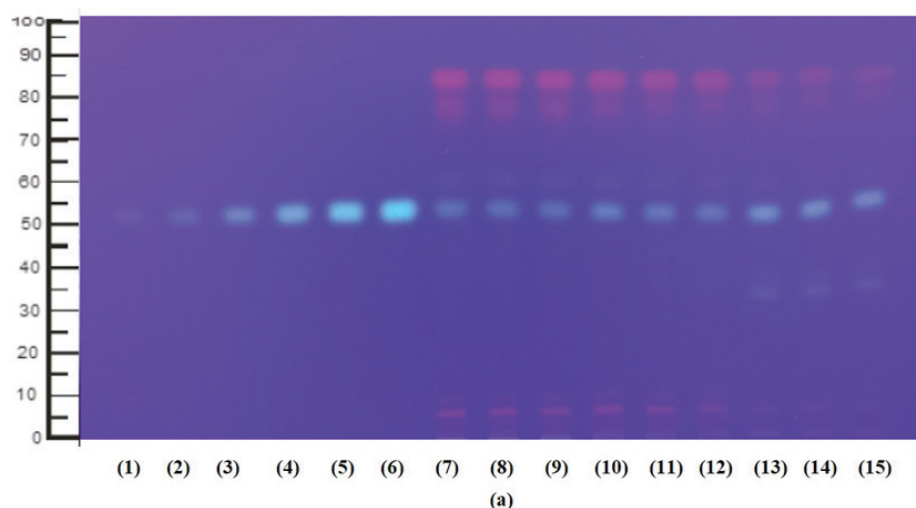


Figure 3. TLC profile of sinensetin and the ethanolic extract of *O. aristatus*. (1–6) Sinensetin, (7–9) ethanolic extract from Suroloyo, (10–12) ethanolic extract from Samigaluh, and (13–15) ethanolic extract from Bagelen.

TLC was conducted on silica gel 60 F₂₅₄ using chloroform–ethyl acetate (6:4 v/v) as the mobile phase

FTIR-based metabolomics and chemometric analyses

Interpretation of IR functional groups

The FTIR spectra and interpretation of functional groups of *O. aristatus* from the three locations are presented in Figure 4 and Table 1, respectively. The spectra were consistent across all samples, showing 11 peaks. The strong peaks were identified at 3281, 1599, and 1020 cm⁻¹, corresponding to alcohol/phenol (–OH (stretching)), alkene (–C=C (stretching)), and alcohol (–CO (stretching)), respectively (Pavia et al., 2015; Shahar et al., 2023). The other peaks repre-

sented alkane (2919, 2850, and 1373 cm⁻¹), aldehyde (1729 cm⁻¹), alcohol (1256 cm⁻¹), and aromatics (812 and 766 cm⁻¹) (Pavia et al., 2015).

An overlay of the FTIR spectra of each sample from the three locations was similar but with different absorbance values (Figure 4.). The peak was different from each other at three locations at 3281 cm⁻¹, that represents alcohol/phenol (–OH (stretching)) (Pavia et al., 2015). Moreover, the FTIR spectra of *O. aristatus* from Samigaluh at peaks of 1599 and 1417 cm⁻¹ were different, which represents –C=C (stretching) and –CH₂ (stretching), respectively (Rohman et al., 2021).

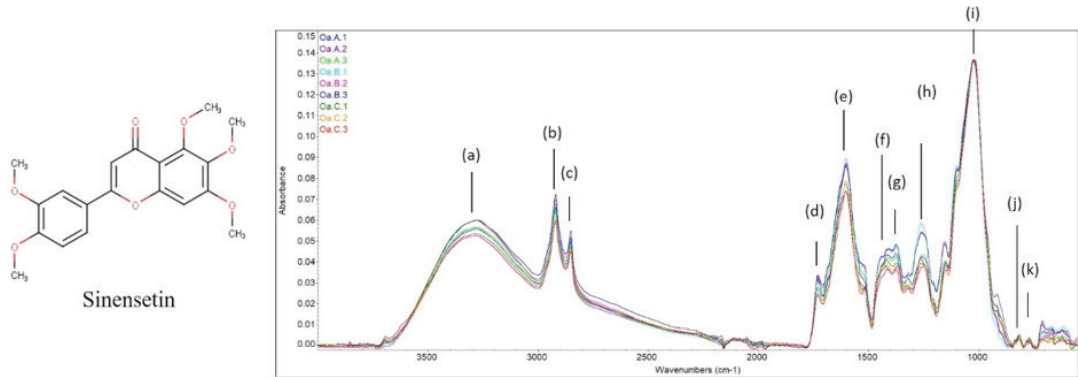


Figure 4. FTIR spectra of *O. aristatus* powder. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

Table 1. Interpretation of the FTIR spectra of *O. aristatus* powder

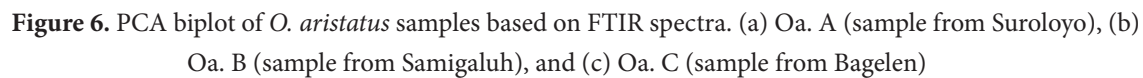
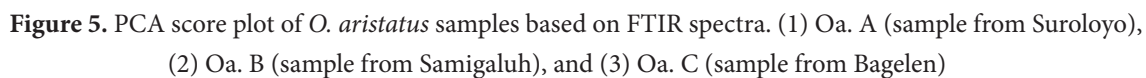
Peak	Measured wavenumbers (cm ⁻¹)	Literature wavenumbers (cm ⁻¹)	Functional groups
(a)	3281	3392 ^[1]	-OH (stretching)
(b)	2919	2927 ^[1]	-CH (stretching)
(c)	2850	2855 ^[1]	-CH (stretching)
(d)	1729	1721 ^[1]	-C=O (stretching)
(e)	1599	1605 ^[2]	-C=C (stretching)
(f)	1417	1416 ^[2]	-CH ₂ (stretching)
(g)	1373	1384 ^[3]	-CH (bend)
(h)	1256	1265 ^[3]	-CO (stretching)
(i)	1020	1030 ^[3]	-CO (stretching)
(j)	812	812 ^[3]	-CH (out-of-plane bend)
(k)	766	536-814 ^[3]	- CH (out-of-plane bend)

[1] Rafi et al., 2015; [2] Rohman et al., 2021; [3] Man et al., 2015

Chemometric analysis of FTIR spectra

Because visual observation alone makes it difficult to discern differences in the FTIR spectra of the *O. aristatus* samples, this study employed chemometric analysis. Two unsupervised chemometrics were used to analyze the FTIR spectra: PCA (score plot and biplot) and CA (dendrogram and heatmap). The score plot and biplot of the PCA analysis are shown in Figures 5 and 6. The PCA score plot model using PC1 (41.6%) and PC2 (37.8%) showed a total variance of 79.4%. PC1–PC5 and PC1–PC4 further explained 95.7% and 92.0% of the total variance, respectively. The PCA model (Figure 5.) showed that all samples were far from each other because of differences in secondary metabolites in the plants.

Moreover, the biplot of the PCA model shows responsive variables in classifying samples. The biplot graph presents the wavenumbers 1020.16 and 992.20 cm⁻¹ essential in classifying samples. Correlation among variables in the biplot graph was evaluated by embedding vectors with origins at PC1 = 0 and PC2 = 0. The angle between vectors can also indicate correlation strength: small angles indicate positive correlation, an angle of about 90° between variables indicates no correlation, and an angle of about 180° between variables indicates a negative correlation (Widodo et al., 2019). For example, the variable at 715.46 cm⁻¹ was positively correlated with that at 1372.07 cm⁻¹, not correlated with that at 1073.19 cm⁻¹, and negatively correlated with that at 951.70 cm⁻¹.



The dendrogram and heatmap of the CA analysis of *O. aristatus* based on FTIR spectra are shown in Figures 7 and 8, respectively. The dendrogram, constructed using Euclidean distances and the Ward clustering system from wavenumbers of samples based on a phylogeny tree, shows the correlation between each sample. The phylogenetic tree divides the samples into three groups according to their origins. This clustering correlates with the secondary metabolites in *O. aristatus*. Meanwhile, the heatmap correlates 29 wavenumbers of all samples using Euclidean distances and the Ward clustering system.

Data in rows and columns represent wavenumbers and samples, respectively. The blue color indicates a decrease in absorbance value. Meanwhile, the red color shows elevated absorbance values. *O. aristatus* samples from Suroloyo had a high absorbance at the wavenumbers of 885.17, 930.49, and 951.70 cm^{-1} . *O. aristatus* samples from Samigaluh had a high absorbance at the wavenumbers 715.46, 1215.90, 1233.26, and 1695.12 cm^{-1} . *O. aristatus* samples from Bagelen had a high absorbance at the wavenumbers of 992.20, 1020.16, 1073.19, 2650.68, 3100.01, and 3380.60 cm^{-1} .

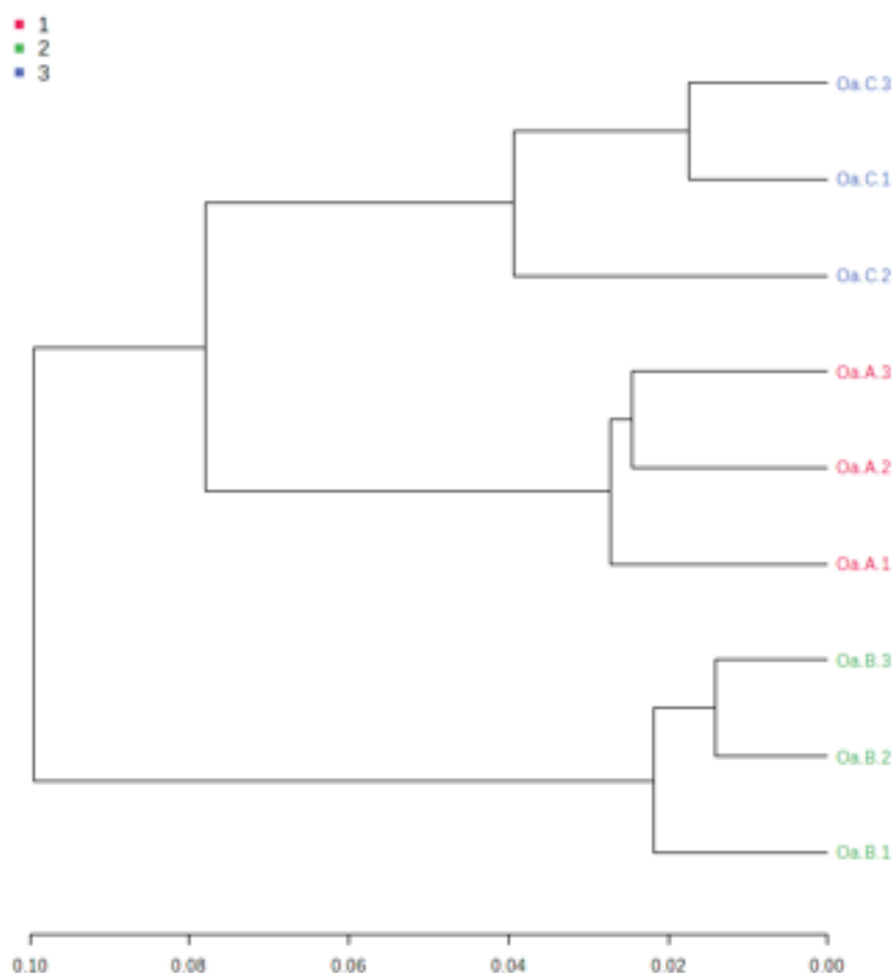


Figure 7. Dendrogram of *O. aristatus* samples based on FTIR spectra. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

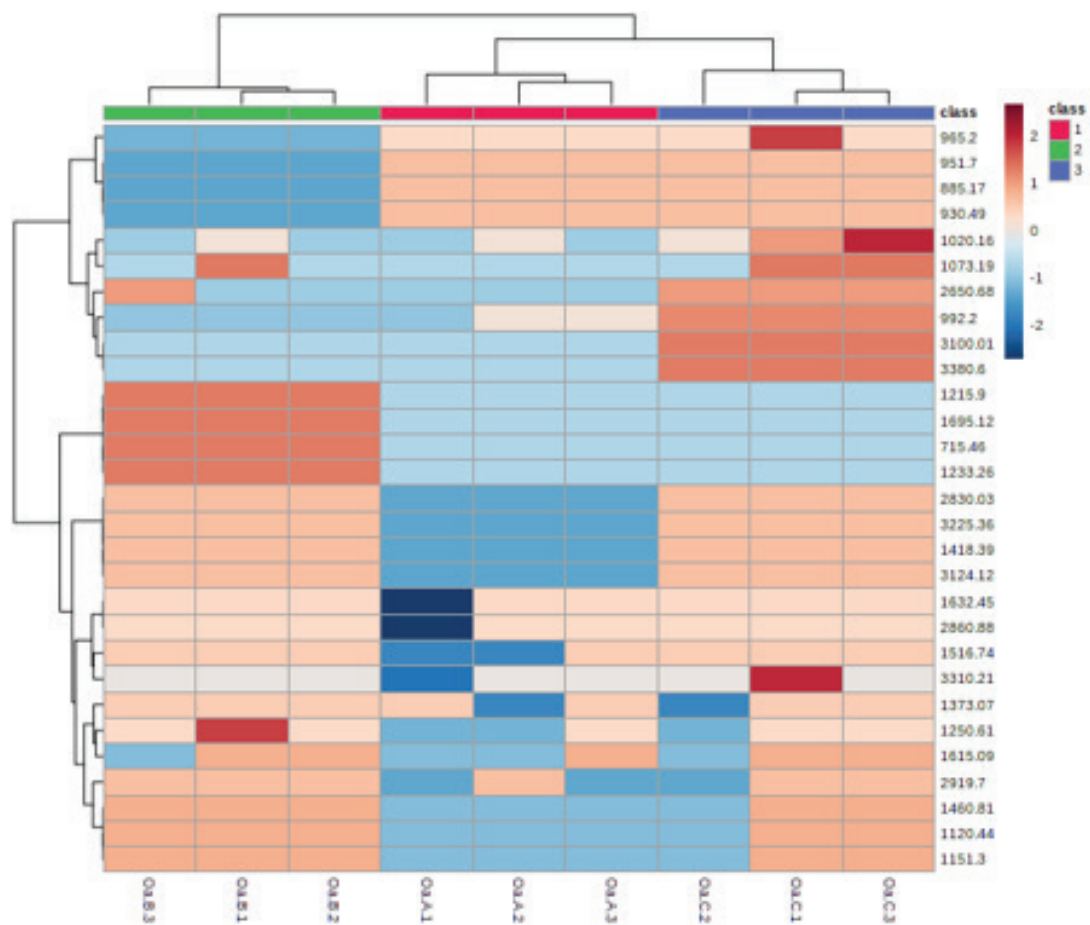


Figure 8. Heatmap of *O. aristatus* samples based on FTIR spectra. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

¹H-NMR-based metabolomics and chemometric analyses

Interpretation of ¹H-NMR spectra

The ¹H-NMR spectra and list of metabolites of *O. aristatus* are shown in Figure 9 and Table 2, respectively. All *O. aristatus* samples had similar ¹H-NMR spectra, indicating similar metabolites. The signals in the ranges of δ 0.5–3.0, δ 3.0–5.5, and δ 5.5–9.0 indicated aliphatic, carbohydrate, and aromatic group com-

pounds, respectively (Tajidin et al., 2019). Although the ¹H-NMR spectra of *O. aristatus* showed the same ppm value, differences in multiplicity were observed compared with previous studies. This could be attributed to overlapping signals caused by the presence of mixed compounds in the extracts (de Falco et al., 2016). *O. aristatus* contained primary and secondary metabolites, such as fatty acids, amino acids, carbohydrates, phenolics, flavonoids, and terpenoids.

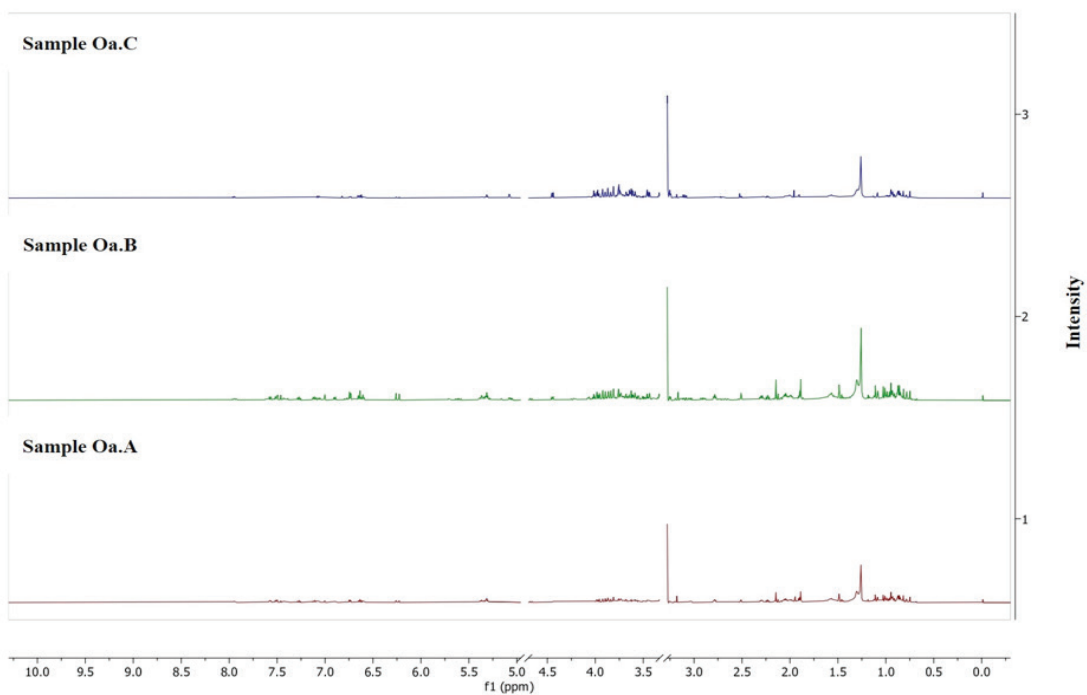


Figure 9. ¹H-NMR spectra of *O. aristatus*. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

Table 2. Interpretation of the ^1H -NMR spectra of *O. aristatus*

No	Chemical class	Chemical compounds	Chemical shift (δ) and multiplicity Oa. A	Chemical shift (δ) and multiplicity Oa. B	Chemical shift (δ) and multiplicity Oa. C	Chemical shift (δ) and multiplicity literature
1	Fatty acids	Myristic acid	1.64 (m)	0.90 (m)*; 1.59 (m)	0.86 (m)*; 1.68 (m)	0.89 (t); 1.64 (m) ^[1]
2	Amino acids	Alanine	3.73 (m)	3.74 (m)	3.73 (m)	3.74 (m) ^[2]
3		Valine	2.27 (m)	2.27 (m)	2.27 (m)	2.29 (m) ^[2]
4		Treonine	-	1.30 (d)*	-	1.32 (t) ^[2]
5		Isoleusine	0.94 (m)*; 1.91 (m); 3.66 (m)*	3.62 (m)*	1.28 (m)	0.92 (t); 1.97 (m); 3.66 (d) ^[1]
6		Glutamine	-	-	2.49 (m)	2.47 (m) ^[2]
7	Carbohydrates	Sucrose	3.87 (m)	3.85 (m)	-	3.86 (m) ^[3]
8	Phenolics	Chlorogenic acid	5.35 (m)	5.35 (m)	5.33 (m)	5.36 (m) ^[2]
9		Rosmarinic acid	3.07 (m)	3.09 (m)	-	3.04 (m) ^[4]
10		Caffeic acid	7.08 (m)*	7.09 (m)*	7.09 (m)*	7.06 (dd) ^[2]
11		Salvianolic acid B	3.07 (m); 6.76 (m)*	3.09 (m); 6.75 (m)*	3.11 (m)	3.06 (m); 6.74 (d) ^[5]
12	Flavonoids	Sinensetin	3.92 (m)*; 6.63 (m)*; 6.76 (m)*; 7.08 (m)*; 7.55 (m)*	3.95 (m)*; 6.62 (m)*; 6.75 (m)*; 7.09 (m)*; 7.50 (m)*	3.98 (m)*; 6.63 (m)*; 7.09 (m)*	3.95 (s); 6.62 (s); 6.72 (s); 7.02 (d); 7.57 (dd) ^[6]
13		Eupatorin	3.79 (m)*; 3.92 (m)*; 7.08 (m)*; 7.45 (m)*; 7.55 (m)*	3.95 (m)*; 6.62 (m)*; 7.09 (m)*; 7.50 (m)*	3.98 (m)*; 6.63 (m)*; 7.09 (m)*	3.98 (s); 3.79 (s); 3.92 (s); 6.67 (s); 7.10 (d); 7.48 (d); 7.55 (dd) ^[7]
14		Luteolin	7.45 (m)*	-	-	7.41 (dd) ^[2]
15		Kaempferol	5.35 (m)*	5.35 (m)*; 6.75 (m)*	4.45 (m); 5.33 (m)	4.50 (d); 5.33 (s); 6.80 (dd) ^[8]
16		Apigenin	6.63 (m)*	6.62 (m)*	6.63 (m)*	6.69 (d) ^[2]
17	Terpenoids	Orthosiphol derivative	2.27 (m)	2.27 (m)	2.26 (m)	2.23 (m) ^[2]

[1] Wishart et al., 2022; [2] Pariyani et al., 2017; [3] Hernández-García et al., 2019 ; [4] Serrano et al., 2021; [5] Sun et al., 2009; [6] Frezza et al., 2021; [7] González-Cortazar et al., 2022; and [8] Smolarz, 2002. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

Chemometric analysis of ^1H -NMR spectra

Because visual observation alone cannot fully discern the differences in the ^1H -NMR spectra of the *O. aristatus* samples, this study employed chemometric analysis. Two unsupervised chemometrics were used to analyze the ^1H -NMR spectra: PCA (score plot and biplot) and CA (dendrogram and heatmap). The score plot and biplot of the PCA analysis based on ^1H -NMR spectra are shown in Figures 10 and 11. The PCA score plot model using PC1 (72.7%) and PC2

(27.3%) showed a total variance of 100%. The PCA model (Figure 10.) showed that all samples were far from each other because of differences in the secondary metabolites in the plants. Furthermore, the PCA biplot showed that bins 1.36532, 1.60629, 3.53402, 2.08822, and 7.50998 were essential in classifying samples. Correlation among variables in the biplot graph was evaluated by embedding vectors with origins at PC1 = 0 and PC2 = 0. For example, the variable at 7.50998 was positively correlated with that at 5.38144 but not with that at 1.60629.

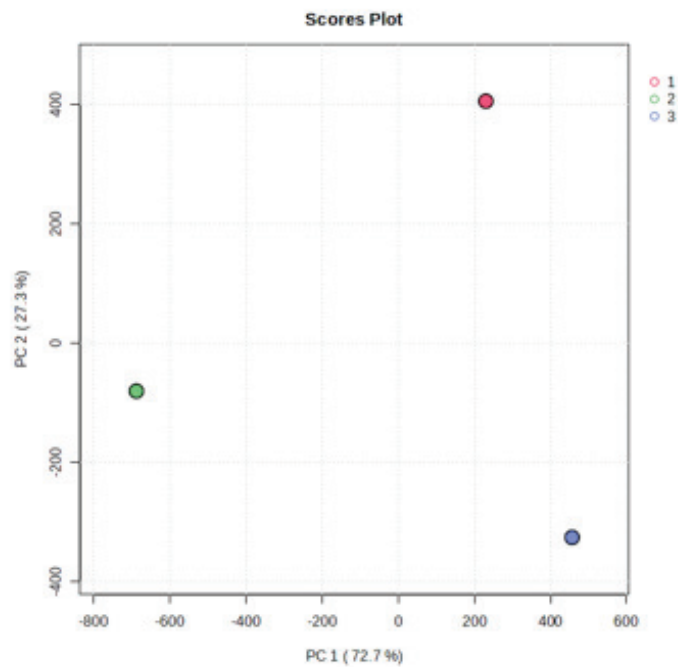


Figure 10. PCA score plot of *O. aristatus* samples based on ¹H-NMR spectra. (1) Oa. A (sample from Suroloyo), (2) Oa. B (sample from Samigaluh), and (3) Oa. C (sample from Bagelen)

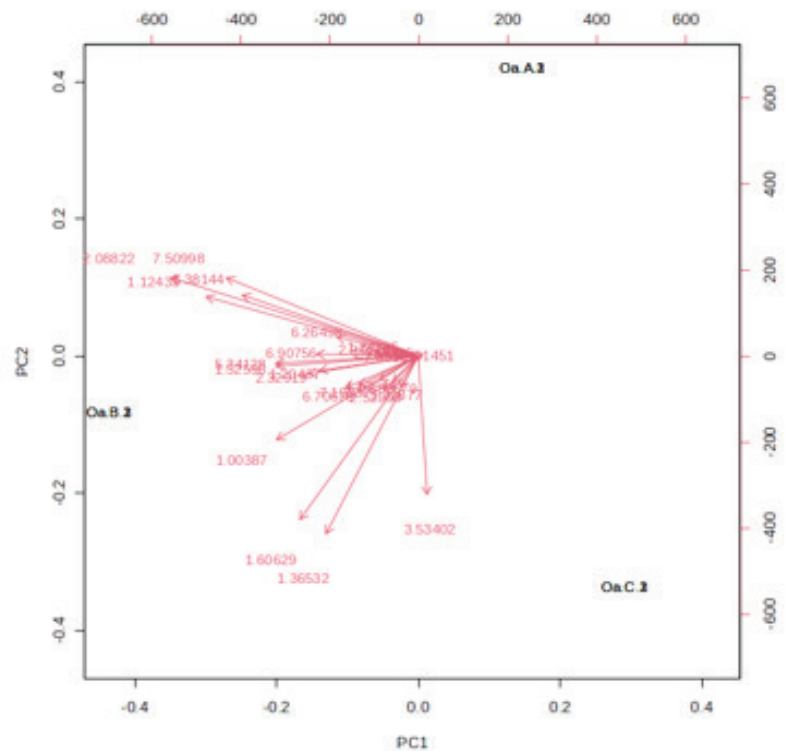


Figure 11. PCA biplot of *O. aristatus* samples based on ¹H-NMR spectra. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

The dendrogram and heatmap of the CA analysis of *O. aristatus* based on ¹H-NMR spectra are shown in Figures 12 and 13. The dendrogram, constructed from the bin values of samples based on a phylogenetic tree using Euclidean distances and the Ward clustering system, shows the correlation between each sample. The phylogenetic tree divides the samples into three groups according to their origins. This clustering correlates with the secondary metabolites in *O. aristatus*. Meanwhile, the heatmap correlates 26 bins of all sam-

ples using Euclidean distances and the Ward clustering system. Data in rows and columns represent bins and samples, respectively. The blue color indicates a decrease in intensity value. Meanwhile, the red color shows elevated intensity values. *O. aristatus* samples from Samigaluh had the highest intensity compared with the samples across all regions. *O. aristatus* samples from Bagelen had the lowest intensity compared with the samples across all regions.

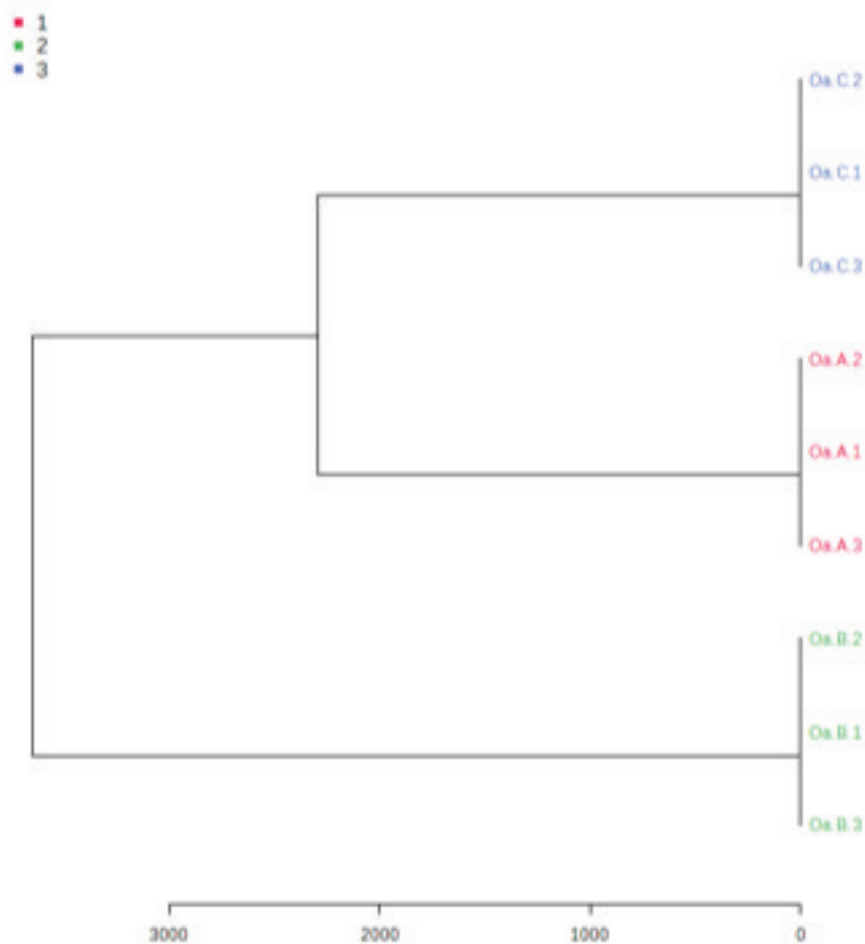


Figure 12. Dendrogram of *O. aristatus* samples based on ¹H-NMR spectra. (a) Oa. A (sample from Suroloyo), (b) Oa. B (sample from Samigaluh), and (c) Oa. C (sample from Bagelen)

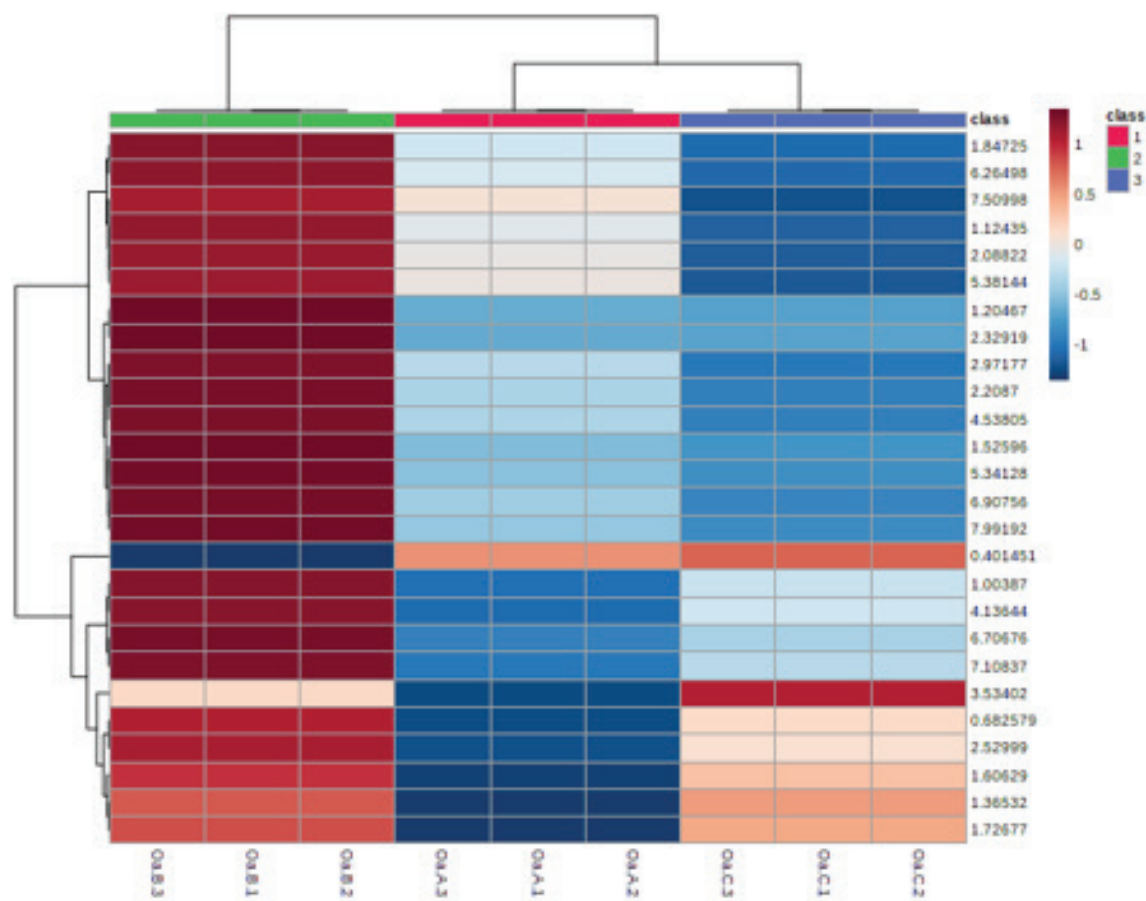


Figure 13. Heatmap of *O. aristatus* samples based on ^1H -NMR spectra. (a) Oa.A (sample from Suroloyo), (b) Oa.B (sample from Samigaluh), and (c) Oa.C (sample from Bagelen)

Each plant has primary and secondary metabolites, which have different biological functions (Hoffmann et al., 2017). Secondary metabolites, also known as phytochemicals (Bai et al., 2011), exhibit diverse applications for human health, with broad pharmacological activities and mechanisms of action (Arifah et al., 2023; Rohman et al., 2020). Many factors influence phytochemical variances, such as environmental conditions (climate [temperature, light, and water], soil, and attack by pathogens and pests) (Hoffmann et al., 2017), geographical origin (Mais et al., 2018), species variations (Liu et al., 2019), postharvest treatment (Pramono et al., 2018), and extraction methods (Song et al., 2021). In this regard, metabolomics provides a tool for measuring such variations. Metabolomics can be used to control the quality of herbal medicine, such

as variety, seasonality, geographical origin, taxonomical identification, extraction method, and authentication (Kharbach et al., 2020). This study reported phytochemical analyses using TLC, FTIR, and ^1H -NMR combined with chemometrics to analyze *O. aristatus* from three locations in Menoreh Hill, Indonesia.

TLC analysis was used to determine the sinensetin level of *O. aristatus* samples from three locations. Before TLC measurement, *O. aristatus* was extracted using 70% v/v ethanol for 24 h. The ethanolic extracts were dark green, odorous, and bitter. These characterizations were similar to those of the ethanolic extract of *O. aristatus* in the Indonesian Herbal Pharmacopoeia (i.e., brown, odorous, and bitter) (Department of Health of the Republic of Indonesia, 2008). The yields of these ethanolic extracts were higher than those re-

quired by the Indonesian Herbal Pharmacopeia (i.e., more than 8.70%) (Department of Health of the Republic of Indonesia, 2008). The ethanolic extract of *O. aristatus* from Samigaluh had the highest yield compared with those from the other locations. However, a high yield does not indicate an active substance level (Pramono & Ajiastuti, 2004). So, this study measured the sinensetin level of the *O. aristatus* samples from all three locations. The ethanolic extracts showed differences in the number of spots on the TLC plate, where the samples from Suroloyo and Samigaluh had seven spots, and those from Bagelen had eight spots. The sinensetin levels of the ethanolic extracts from the three locations were smaller than the sinensetin level in the Indonesian Herbal Pharmacopeia (i.e., not less than 1.10% w/w) (Department of Health of the Republic of Indonesia, 2008). This result was similar to that of Kartini's study, which found that sinensetin levels from 14 locations in East Java and other regions in Indonesia were below the required sinensetin level in the Indonesian Herbal Pharmacopeia (Kartini et al., 2022).

In addition, this study analyzed untargeted metabolomics using FTIR and ¹H-NMR spectroscopy combined with chemometrics. The samples from all three locations had similar FTIR and ¹H-NMR spectra, indicating similar secondary metabolites. Chemometrics was employed to classify *O. aristatus* from all three locations based on the FTIR and ¹H-NMR spectra. PCA of the FTIR spectra distinguished the samples, with wavenumbers 1020.16 and 992.20 cm⁻¹ playing an essential role in sample classification. Similarly, PCA of the ¹H-NMR spectra identified bins 1.36532, 1.60629, 3.53402, 2.08822, and 7.50998 as having important roles in sample classification. Meanwhile, CA (dendrogram and heatmap) of *O. aristatus* powder based on FTIR and ¹H-NMR spectra showed that the samples from Suroloyo and Bagelen were more closely related compared with the samples from Samigaluh. The phytochemical profiles of *O. aristatus* according to TLC, FTIR, and ¹H-NMR revealed differences in both types and levels of secondary me-

tabolites, which could be attributed to variations in geographic origin (i.e., different growing altitudes) (Peñaloza et al., 2018). Differences in altitude impact various environmental conditions, including the variance of secondary metabolites (Hoffmann et al., 2017).

Several studies from previous reports revealed a similarity of phytochemical constituents of *O. aristatus* with the recent study. Aerial parts of *O. aristatus* from Thailand showed four flavonoids (sinensetin, salvigenin, tetramethylscutellarein, and 3,7,4'-tri-O-methylkaempferol) and diterpenoids (orthosiphol A) (Damsud et al., 2014). A recent study showed various metabolite secondary compounds based on ¹H-NMR spectra, such as phenolics (chlorogenic acid, rosmarinic acid, caffeic acid, and salvianolic acid B), flavonoids (sinensetin, eupatorin, luteolin, kampferol, and apigenin), and terpenoids (orthosiphol derivatives). Thus, a study from China showed that several phytochemical compounds, which are similar to those in the recent study, such as phenolics (chlorogenic acid, rosmarinic acid, caffeic acid, and salvianolic acid B) and flavonoids (sinensetin and eupatorin) (Ouyang et al., 2024). Another report from Indonesia (Yogyakarta) reports three phenolics, including chlorogenic acid, caffeic acid, and rosmarinic acid (Ahda et al., 2025).

CONCLUSION

Targeted and untargeted metabolomics studies, utilizing TLC, FTIR, and ¹H-NMR, combined with chemometric analyses, were employed to determine metabolite differences in three geographical locations on Menoreh Hill, Indonesia. The sinensetin levels based on TLC analyses of *O. aristatus* samples from Suroloyo, Samigaluh, and Bagelen were 0.044% ± 0.000% w/w, 0.075% ± 0.002% w/w, and 0.109% ± 0.001% w/w, respectively. Samples from Suroloyo and Samigaluh showed seven spots, while samples from Bagelen showed eight spots. Thus, the *O. aristatus* sample from Suroloyo was more similar to that from Bagelen than to that from Samigaluh, as indicated by the FTIR and ¹H-NMR spectra. FTIR has demonstrated its ability to predict the presence of

phytochemical compounds in *O. aristatus*, including phenols, alcohols, alkanes, alkenes, aldehydes, and aromatics. FTIR, combined with chemometrics, is a straightforward, cost-effective, and environmentally friendly tool for discriminating and classifying *O. aristatus* samples from different geographical locations. ¹H-NMR was able to monitor the phytochemical profiles, involving six classes (fatty acids, amino acids, carbohydrates, phenolics, flavonoids, and terpenoids). ¹H-NMR, combined with chemometrics, is suitable for metabolomics applications involving medicinal plants from different geographical origins. Finally, the phytochemical profiles gained from the analytical instruments in this study can be used in future *O. aristatus* research.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia [Grant No. 4454/UN1/DITLIT/DIT-LIT/PT/2021] through the scheme of the research project of Pendidikan Magister Menuju Doktor untuk Sarjana Unggul (PMDSU).

AUTHOR CONTRIBUTION STATEMENT

FHA: Conceptualization, Methodology, Formal Analysis, Data Curation, Writing – Original Draft, Writing – Review & Editing. **AR:** Conceptualization, Methodology, Supervision, Writing – Review & Editing. **WS:** Supervision, Writing – Review & Editing. **AEN:** Conceptualization, Supervision, Writing – Review & Editing. All the authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adhikary, S., & Dasgupta, N. (2023). Role of secondary metabolites in plant homeostasis during biotic stress. *Biocatalysis and Agricultural Biotechnology*, 50, 102712. <https://doi.org/10.1016/j.bcab.2023.102712>

Ahda, M., Jaswir, I., Khatib, A., Ahmed, Q. U., Mahfudh, N., Prastowo, I.,...Samdani, M. S. (2025). Quality Control of the Various *Orthosiphon stamineus* Leaf Extracts Using High-Performance Liquid Chromatography: Multivariate-Based and Marker-Based Analytical Techniques. *Food Analytical Methods*, 18(4), 552–563. <https://doi.org/10.1007/s12161-024-02738-y>

Akowiuh, G. A., Zhari, I., Sadikun, A., & Norhayati, I. (2006). HPTLC Densitometric Analysis of *Orthosiphon stamineus*. Leaf Extracts and Inhibitory Effect on Xanthine Oxidase Activity. *Pharmaceutical Biology*, 44(1), 65–70. <https://doi.org/10.1080/13880200500531045>

Arifah, F. H., Nugroho, A. E., Rohman, A., & Sujarwo, W. (2022). A review of medicinal plants for the treatment of diabetes mellitus: The case of Indonesia. *South African Journal of Botany*, 149, 537–558. <https://doi.org/https://doi.org/10.1016/j.sajb.2022.06.042>

Arifah, F. H., Nugroho, A. E., Rohman, A., & Sujarwo, W. (2023). A Bibliometric Analysis to Pre-clinical Studies of *Tinospora crispa* (L.) Hook. F. & Thomson as An Antidiabetic. *Indonesian Journal of Pharmacy*, 34(1), 24–35. <https://doi.org/https://doi.org/10.22146/ijp.4963>

Arifah, F. H., Nugroho, A. E., Rohman, A., & Sujarwo, W. (2025). Characterization of biochemical and histological of fructose-based high-fat diet and low-dose streptozotocin in type 2 diabetes mellitus rat model. *Indonesian Journal of Pharmacy*, 36(1), 82–95. <https://doi.org/https://doi.org/10.22146/ijp.6303>

Arifah, F. H., Woerdenbag, H. J., & Fakhrudin, N. (2026). FTIR spectroscopy and chemometrics for chemotaxonomy of Zingiberaceae species indigenous to Indonesia. *South African Journal of Botany*, 189, 243–251. <https://doi.org/10.1016/j.sajb.2025.11.044>

- Azahari, N., Khattak, M. M. A. K., Taher, M., & Ichwan, S. J. A. (2015). Herbal extracts exhibit anti-diabetic activities in 3T3-L1 adipocytes model. *Progress in Nutrition*, 17(4), 301–310.
- Bai, F. W., Zhao, X.-Q., & Xu, J. (2011). Immobilization Technology. In M. Moo-Young (Ed.), *Comprehensive Biotechnology* (pp. 477–489). Elsevier. <https://doi.org/10.1016/B978-0-08-088504-9.00115-X>
- Çiçek, S. S., Mangoni, A., Hanschen, F. S., Agerbirk, N., & Zidorn, C. (2024). Essentials in the acquisition, interpretation, and reporting of plant metabolite profiles. *Phytochemistry*, 220, 114004. <https://doi.org/10.1016/j.phytochem.2024.114004>
- Cuadros-Rodríguez, L., Ruiz-Samblás, C., Valverde-Som, L., Pérez-Castaño, E., & González-Casado, A. (2016). Chromatographic fingerprinting: An innovative approach for food “identification” and food authentication – A tutorial. *Analytica Chimica Acta*, 909, 9–23. <https://doi.org/10.1016/j.aca.2015.12.042>
- Damsud, T., Grace, M. H., Adisakwattana, S., & Phuwapraisirisan, P. (2014). Orthosiphon A from the aerial parts of *Orthosiphon aristatus* is putatively responsible for hypoglycemic effect via α -glucosidase inhibition. *Natural Product Communications*, 9(5), 1934578X1400900. <https://doi.org/10.1177/1934578X1400900512>
- de Falco, B., Incerti, G., Pepe, R., Amato, M., & Lanzotti, V. (2016). Metabolomic Fingerprinting of Romaneschi Globe Artichokes by NMR Spectroscopy and Multivariate Data Analysis: Metabolomics of Artichokes by NMR and Chemometrics. *Phytochemical Analysis*, 27(5), 304–314. <https://doi.org/10.1002/pca.2632>
- Department of Health of the Republic of Indonesia. (2008). *Indonesian Herbal Pharmacopoeia* (I). Department of Health of the Republic of Indonesia.
- Ellis, D. I., & Goodacre, R. (2012). Metabolomics-assisted synthetic biology. *Current Opinion in Biotechnology*, 23(1), 22–28. <https://doi.org/https://doi.org/10.1016/j.copbio.2011.10.014>
- Faramayuda, F., Riyanti, S., Pratiwi, A. S., Mariani, T. S., Elfahmi, E., & Sukrasno, S. (2021). Isolasi Sinensetin dari Kumis Kucing (*Orthosiphon aristatus* Blume miq.) Varietas Putih. *JPSCR: Journal of Pharmaceutical Science and Clinical Research*, 6(2), 111–127. <https://doi.org/10.20961/jpscr.v6i2.48084>
- Fatmarahmi, D. C., Susidarti, R. A., Swasono, R. T., & Rohman, A. (2022). A development method of FTIR spectroscopy coupled with chemometrics for detection of synthetic drug adulterants of herbal products in quaternary mixture. *Journal of Applied Pharmaceutical Science*, 12(3), 191–201. <https://doi.org/10.7324/JAPS.2022.120320>
- Frezza, C., Venditti, A., De Vita, D., Sciubba, F., Tomai, P., Franceschin, M.,...Bianco, A. (2021). Phytochemical Analysis and Biological Activities of the Ethanolic Extract of *Daphne sericea* Vahl Flowering Aerial Parts Collected in Central Italy. *Biomolecules*, 11(3), 379. <https://doi.org/10.3390/biom11030379>
- González-Cortazar, M., Salinas-Sánchez, D. O., Herrera-Ruiz, M., Román-Ramos, D. C., Zamilpa, A., Jiménez-Ferrer, E.,...Pérez-García, Ma. D. (2022). Eupatorin and Salviandulin-A, with Antimicrobial and Anti-Inflammatory Effects from *Salvia lavanduloides* Kunth Leaves. *Plants*, 11(13), 1739. <https://doi.org/10.3390/plants11131739>
- Hamadi, N., Mansour, A., Hassan, M. H., Khalifi-Touhami, F., & Badary, O. (2012). Ameliorative effects of resveratrol on liver injury in streptozotocin-induced diabetic rats. *Journal of Biochemical and Molecular Toxicology*, 26(10), 384–392. <https://doi.org/10.1002/jbt.21432>

- Hashim, S., Beh, H., Hamil, M. R., Ismail, Z., & Majid, A. S. A. (2016). High-performance thin-layer chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of *Orthosiphon stamineus*. *Pharmacognosy Research*, 8(4), 238. <https://doi.org/10.4103/0974-8490.188872>
- Hernández-García, E., García, A., Avalos-Alanís, F. G., Rivas-Galindo, V. M., Delgadillo-Puga, C., & Camacho-Corona, M. del R. (2019). Nuclear magnetic resonance spectroscopy data of isolated compounds from *Acacia farnesiana* (L) Willd fruits and two esterified derivatives. *Data in Brief*, 22, 255–268. <https://doi.org/10.1016/j.dib.2018.12.008>
- Hoffmann, J. F., Carvalho, I. R., Barbieri, R. L., Romaldi, C. V., & Chaves, F. C. (2017). *Butia* spp. (Arecaceae) LC-MS-Based Metabolomics for Species and Geographical Origin Discrimination. *Journal of Agricultural and Food Chemistry*, 65(2), 523–532. <https://doi.org/10.1021/acs.jafc.6b03203>
- Indariani, S., Wijaya, H., Rahminiwati, M., & Winarno, W. (2014). Antihyperglycemic activity of functional drinks based on Java Tea (*Orthosiphon aristatus*) in streptozotocin induced diabetic mice. *International Food Research Journal*, 21(1), 349–355.
- Kartini, K., Dewi, E. R., Achmad, F., Eka Jayani, N. I., Arbi Hadiyat, M., & Avanti, C. (2020). Thin Layer Chromatography Fingerprinting and Clustering of *Orthosiphon stamineus* Benth. From Different Origins. *Pharmacognosy Journal*, 12(1), 79–87. <https://doi.org/10.5530/pj.2020.12.13>
- Kartini, K., Putri, R. E., & Budiono, R. (2022). Quantification of sinensetin in *Orthosiphon stamineus* from various phytogeographical zones in Indonesia. *Journal of Applied Pharmaceutical Science*, 13(3), 183–191. <https://doi.org/10.7324/JAPS.2023.80035>
- Kementerian Kesehatan Republik Indonesia. (2017). *Keputusan Menteri Kesehatan Republik Indonesia Nomor Hk.01.07/Menkes/187/2017 Tentang Formularium Ramuan Obat Tradisional Indonesia*. Kementerian Kesehatan Republik Indonesia.
- Kharbach, M., Marmouzi, I., El Jemli, M., Bouklouze, A., & Vander Heyden, Y. (2020). Recent advances in untargeted and targeted approaches applied in herbal-extracts and essential-oils fingerprinting—A review. *Journal of Pharmaceutical and Biomedical Analysis*, 177, 112849. <https://doi.org/10.1016/j.jpba.2019.112849>
- Lee, H.-J., Choi, Y.-J., Park, S.-Y., Kim, J.-Y., Won, K.-C., Son, J.-K., & Kim, Y.-W. (2015). Hexane Extract of *Orthosiphon stamineus* Induces Insulin Expression and Prevents Glucotoxicity in INS-1 Cells. *Diabetes & Metabolism Journal*, 39(1), 51–58. <https://doi.org/10.4093/dmj.2015.39.1.51>
- Liu, F., Meng, Y., He, K., Song, F., Cheng, J., Wang, H.,...Yan, X. (2019). Comparative analysis of proteomic and metabolomic profiles of different species of Paris. *Journal of Proteomics*, 200, 11–27. <https://doi.org/10.1016/j.jprot.2019.02.003>
- Mais, E., Alolga, R. N., Wang, S.-L., Linus, L. O., Yin, X., & Qi, L.-W. (2018). A comparative UPLC-Q/TOF-MS-based metabolomics approach for distinguishing *Zingiber officinale* Roscoe of two geographical origins. *Food Chemistry*, 240, 239–244. <https://doi.org/10.1016/j.foodchem.2017.07.106>
- Man, S., Sui Kiong, L., Ab'lah, N. A., & Abdullah, Z. (2015). Differentiation of the white and purple flower forms of *Orthosiphon aristatus* (Blume) miq. By 1D and 2D correlation IR spectroscopy. *Jurnal Teknologi*, 77(3), 81–86. <https://doi.org/10.11113/jt.v77.6010>

- Mardiswojo, S., & Rajakmangunsudarso, H. (1987). *Cabe Puyang Warisan Nenek Moyang*. Balai Pustaka.
- Markley, J. L., Bruschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., & Wishart, D. S. (2017). The future of NMR-based metabolomics. *Current Opinion in Biotechnology*, 43, 34–40. <https://doi.org/https://doi.org/10.1016/j.copbio.2016.08.001>
- Maulana, F., Muhammad, A. A., Umar, A., Mahendra, F. R., Musthofa, M., & Nurcholis, W. (2022). Profiling Metabolites through Chemometric Analysis in *Orthosiphon aristatus* Extracts as α -Glucosidase Inhibitory Activity and *In Silico* Molecular Docking. *Indonesian Journal of Chemistry*, 22(1), 501–514. <https://doi.org/10.22146/ijc.71334>
- McMillen, T. S., Leslie, A., Chisholm, K., Penny, S., Gallant, J., Cohen, A.,...Pinto, D. M. (2023). A large-scale, targeted metabolomics method for the analysis and quantification of metabolites in human plasma via liquid chromatography-mass spectrometry. *Analytica Chimica Acta*, 1279, 341791. <https://doi.org/10.1016/j.aca.2023.341791>
- Mohamed, E. A. H., Mohamed, A. J., Asmawi, Mohd. Z., Sadikun, A., Ebrika, O. S., & Yam, M. F. (2011). Antihyperglycemic effect of *Orthosiphon stamineus* Benth leaves extract and its bioassay-guided fractions. *Molecules*, 16(5), 3787–3801. <https://doi.org/10.3390/molecules16053787>
- Mohamed, E. A. H., Siddiqui, M. J. A., Ang, L. F., Sadikun, A., Chan, S. H., Tan, S. C.,...Yam, M. F. (2012). Potent α -glucosidase and α -amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. *BMC Complementary and Alternative Medicine*, 12(1), 176. <https://doi.org/10.1186/1472-6882-12-176>
- Nguyen, P. H., Tuan, H. N., Hoang, D. T., Vu, Q. T., Pham, M. Q., Tran, M. H., & To, D. C. (2019). Glucose uptake stimulatory and PTP1B inhibitory activities of pimarane diterpenes from *Orthosiphon stamineus* Benth. *Biomolecules*, 9(12), 859. <https://doi.org/10.3390/biom9120859>
- Onuh, J. O., & Aluko, R. E. (2019). Metabolomics as a tool to study the mechanism of action of bioactive protein hydrolysates and peptides: A review of current literature. *Trends in Food Science & Technology*, 91, 625–633. <https://doi.org/https://doi.org/10.1016/j.tifs.2019.08.002>
- Ouyang, J., Lin, D., Chen, X., Li, Y., Liu, Q., Li, D.,... Mao, W. (2024). Analysis of the chemical constituents and their metabolites in *Orthosiphon stamineus* Benth. Via UHPLC-Q exactive orbitrap-HRMS and AFADESI-MSI techniques. *PLOS ONE*, 19(6), e0304852. <https://doi.org/10.1371/journal.pone.0304852>
- Pariyani, R., Ismail, I. S., Ahmad Azam, A., Abas, F., & Shaari, K. (2017). Identification of the compositional changes in *Orthosiphon stamineus* leaves triggered by different drying techniques using ¹ H NMR metabolomics: Metabolite profiling of *Orthosiphon stamineus* leaves. *Journal of the Science of Food and Agriculture*, 97(12), 4169–4179. <https://doi.org/10.1002/jsfa.8288>
- Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. R. (2015). *Introduction to Spectroscopy: A Guide for Students of Organic Chemistry* (5th ed.). Cengage Learning.
- Peñaloza, E., Casanova, L., Leal, I., de Aguiar, P., & Costa, S. (2018). Metabolite Fingerprinting and Profiling of the Medicinal Grass *Eleusine indica* Based on HPLC-DAD, UPLC-DAD-MS/MS and NMR Analyses. *Journal of the Brazilian Chemical Society*. <https://doi.org/10.21577/0103-5053.20180131>

- Prajapati, P. B., Patel, P. R., & Shah, S. A. (2022). Chemometric and DoE-Based Analytical Quality Risk Management to HPTLC Method for Simultaneous Estimation of Metronidazole and Norfloxacin. *Journal of Chromatographic Science*, 61(5), 428–439. <https://doi.org/10.1093/chromsci/bmab145>
- Pramono, S., & Ajiastuti, D. (2004). Standardisasi ekstrak herba pegagan (*Centella asiatica*.(L.).Urban) berdasarkan kadar asiatikosida secara KLT-densitometri. *Majalah Farmasi Indonesia*, 15(3), 118–123.
- Pramono, S., Arifah, F. H., Pribadi, F. H., & Nugroho, A. E. (2018). Hepatoprotective activity of *Curcuma xanthorrhiza* Roxb. On paracetamol-induced liver damage in rats and correlation with their chemical compounds. *Thai Journal of Pharmaceutical Sciences*, 42(4), 188–195. <https://doi.org/10.56808/3027-7922.2366>
- Rafi, M., Purwakusumah, E. D., Ridwan, T., Barus, B., Sutandi, A., & Darusman, L. K. (2015). Geographical classification of Java Tea (*Orthosiphon stamineus*) from Java Island by FTIR Spectroscopy Combined with Canonical Variate Analysis. *Jurnal Sains Dan Matematika*, 23(1), 25–31.
- Rao, N. K., Bethala, K., Sisinthy, S. P., & Rajeswari, S. (2014). Antidiabetic activity of *Orthosiphon stamineus* Benth roots in streptozotocin induced type 2 diabetic rats. *Asian Journal of Pharmaceutical and Clinical Research*, 7(1), 49–153.
- Rohman, A., Arifah, F. H., Irnawati, Alam, G., Muchtaridi, & Rafi, M. (2020). A review on phytochemical constituents, role on metabolic diseases, and toxicological assessments of underutilized part of *Garcinia mangostana* L. fruit. *Journal of Applied Pharmaceutical Science*, 10(7), 127–146. <https://doi.org/10.7324/JAPS.2020.10716>
- Rohman, A., Luthfianasari, H., Irnawati, I., Riyanto, S., Rafi, M., Prajogo, B., & Amran, M. B. (2021). HPLC-FTIR spectroscopy combined with multivariate calibration for analysis of Andrographolide in *Andrographis paniculata* extract. *Journal of Applied Pharmaceutical Science*, 11(5). <https://doi.org/10.7324/JAPS.2021.110505>
- Serrano, C. A., Villena, G. K., & Rodríguez, E. F. (2021). Phytochemical profile and rosmarinic acid purification from two Peruvian *Lepechinia* Willd. species (Salviinae, Mentheae, Lamiaceae). *Scientific Reports*, 11(1), 7260. <https://doi.org/10.1038/s41598-021-86692-3>
- Shah, S. M. Z., Ramzan, M., Khan, M. N., Shadab, H., Usman, M., Rahman, S.,...Musharraf, S. G. (2023). Untargeted screening of plant metabolites based on data-independent and data-dependent acquisition modes using LC-ESI-QTOF-MS: *Tribulus terrestris* L. as a case study. *Arabian Journal of Chemistry*, 16(8), 104978. <https://doi.org/10.1016/j.arabjc.2023.104978>
- Shahar, B., Dolma, N., & Chongtham, N. (2023). Phytochemical analysis, antioxidant activity and identification of bioactive constituents from three wild medicinally important underutilized plants of Ladakh, India using GCMS and FTIR based metabolomics approach. *Food and Humanity*, 1, 430–439. <https://doi.org/10.1016/j.foohum.2023.06.022>
- Smolarz, H. D. (2002). Flavonoids from *Polygonum lapathifolium* ssp. *Tomentosum*. *Pharmaceutical Biology*, 40(5), 390–394. <https://doi.org/10.1076/phbi.40.5.390.8455>
- Song, E., Choi, J., Gwon, H., Lee, K.-Y., Choi, S.-G., Atiqul Islam, Md.,...Hwang, J. (2021). Phytochemical profile and antioxidant activity of *Dracocephalum moldavica* L. seed extracts using different extraction methods. *Food Chemistry*, 350, 128531. <https://doi.org/10.1016/j.foodchem.2020.128531>

- Sriplang, K., Adisakwattana, S., Rungsipipat, A., & Yibchok-anun, S. (2007). Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 109(3), 510–514. <https://doi.org/10.1016/j.jep.2006.08.027>
- Sun, Y., Zhu, H., Wang, J., Liu, Z., & Bi, J. (2009). Isolation and purification of salvianolic acid A and salvianolic acid B from *Salvia miltiorrhiza* by high-speed counter-current chromatography and comparison of their antioxidant activity. *Journal of Chromatography B*, 877(8–9), 733–737. <https://doi.org/10.1016/j.jchromb.2009.02.013>
- Tajidin, N. E., Shaari, K., Maulidiani, M., Salleh, N. S., Ketaren, B. R., & Mohamad, M. (2019). Metabolite profiling of *Andrographis paniculata* (Burm. F.) Nees. Young and mature leaves at different harvest ages using 1H NMR-based metabolomics approach. *Scientific Reports*, 9(1), 16766. <https://doi.org/10.1038/s41598-019-52905-z>
- United States Department of Agriculture. (2023). *Classification for Kingdom Plantae Down to Species Orthosiphon aristatus (Blume) Miq.* <https://plants.usda.gov/images/plants-logo-transparent.png>
- Widodo, H., Sismindari, S., Asmara, W., & Rohman, A. (2019). Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *Journal of Applied Pharmaceutical Science*, 9(6), 99–105. <https://doi.org/10.7324/JAPS.2019.90614>
- Wishart, D. S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., Dizon, R., Sayeeda, Z., Tian, S., Lee, B. L., Berjanskii, M., Mah, R., Yamamoto, M., Jovel, J., Torres-Calzada, C., Hiebert-Giesbrecht, M., Lui, V. W., Varshavi, D., Varshavi, D., ... Gautam, V. (2022). HMDB 5.0: The Human Metabolome Database for 2022. *Nucleic Acids Research*, 50(D1), D622–D631. <https://doi.org/10.1093/nar/gkab1062>
- Yong, X., Li, J., Han, S., Yang, X., Du, J., Hou, Z.,...Xu, H. (2025). Authentication of *Orthosiphon aristatus* and its Common Adulterants Using Integrative Strategy Combined Untargeted Metabolomics with Feature-Based Molecular Network. SSRN. <https://doi.org/10.2139/ssrn.5093201>