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Simple Tool of Metabolomic Studies: TLC-Based Metabolite Profiling *Peronema canescens* Jack in Archipelago Indonesia

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ABSTRACT

Peronema canescens Jack is a species of tropical plant utilized for its empirical applications, such as an antipyretic, antimalarial, immune stimulant, cold medicine, and mouthwash for its antiseptic properties. The objective of this study was to identify differences in the metabolite composition of *P. canescens* Jack leaves at different geographical locations. The metabolite profile was examined by thin-layer chromatography densitometry. The samples were collected from 10 distinct locations at varying elevations, with each location being sourced from three separate plants. Fingerprint metabolomics employs chemometric techniques such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The PCA analysis resulted in a PC1 value of 41% and a PC2 value of 32.6%, and these findings indicate the importance of considering the geographical locations where leaves are collected, as they reveal regional differences in the metabolism of *P. canescens* Jack secondary metabolites. Hierarchical cluster analysis (HCA) classified *P. canescens* Jack into four distinct clusters based on the closeness of their metabolite profiles. TLC is useful for the quality control of *P. canescens* Jack leaves based on the variation geographical and authentication.

Keywords: chemometrics; *Peronema canescens* Jack; PCA; TLC; metabolomics

INTRODUCTION

Peronema canescens Jack also known as *sungkai* or *jati sabrang* plant in Indonesia. It is a tropical plant indigenous to Thailand, Indonesia, and Malaysia (Putranto, 2014) and can be found growing naturally in both lowland and highland forests or gardens. *P. canescens* Jack leaves have bioactive compounds that play as anti-inflammatory (Latief, Anggun, et al., 2021; Tarigan et al., 2022), anti-malarial, antipyretic (Putranto, 2014), antidiabetic (Latief, Sari, et al., 2021), and immunomodulatory (Dillasamola et al., 2021). Many secondary metabolites, including peronemin A2, B1, B2, C1, A3, D1, β Amyrin, Phytol, β Sitosterol (Tarigan et al., 2022), apigenin, betulinic acid, and stigmasterol (Muharni et al., 2021), are found in *P. canescens* Jack leaves.

Genetic, morphogenetic, ontogenic, and environmental factors can influence the presence of secondary metabolites in plants. The variability of environmental conditions in different regions is a contributing factor to the impact of geographic variation on the synthesis of secondary metabolites (Verma & Shukla, 2015). Environmental conditions and plant biosynthesis processes interact to produce chemical components of plants that are also influenced by the altitude of the terrain (Demasi et al., 2018). The abundance of chemical components in a plant of a particular species can be influenced by fluctuations in its growing area (Demasi et al., 2018; Verma & Shukla, 2015).

Extensive research has been conducted on the fingerprinting of plant metabolism by a variety of techniques, such as thin-layer chromatographic (TLC) densitometry (Mayasari et al., 2022; Mutiah et al., 2019; Zahiruddin et al., 2021). The TLC densitometry-based metabolomic method is utilized to determine the chemical composition quantification from the phytochemical profile. Phytochemical profiles provide an indication of the distinct chemical makeup exhibited by individual plants. The World Health Organization concurs that chromatography fingerprint analysis may be utilized to verify the quality of a traditional medicinal product (WHO, 2000). A domain within metabolomic science known as "metabolite fingerprinting" finds application across diverse sectors to fulfill specific objectives. Metabolic fingerprinting is a big part of metabolomics, and it is constantly being used in new areas of study, like

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finding new drugs from natural sources and making sure the quality of herbal materials (authentication) (Yunita et al., 2019). These also include identifying and tracing the origin of plants, ensuring the quality of traditional medicine products, and detecting counterfeit substances in similar products (Nurani et al., 2021).

The development of metabolomics applications, apart from thin-layer chromatography (TLC) (Kartini et al., 2023), has used other methodologies such as High-Performance Thin-Layer Chromatography (HPTLC) (Mayasari et al., 2022; Mutiah et al., 2019; Salomé-Abarca et al., 2021), UV-vis spectrophotometry, Fourier Transform Infrared (FT IR) (Rohaeti et al., 2021), GC-MS, HPLC (Carvalho et al., 2021), Nuclear Magnetic Resonance (NMR) (Nurani et al., 2021), and Liquid Chromatography – High-Resolution Mass Spectrometry (LC HRMS) (Klau et al., 2023). These techniques have the capability of identifying variations in the chemical components of plants more accurately and sensitively. With NMR and LC/MS advanced methods and high-throughput-based metabolomics (Nagana Gowda & Raftery, 2023; Plumb et al., 2023), large amounts of data are generated from the results of chemical components by different procedures. The data will be very much in the form of signals and chromatograms, which are more complicated to process into data sheets and perform multivariate analysis. These data are then processed into data sheets and subjected to multivariate analysis. This method is a valuable instrument for analyzing large data sets, particularly those containing high-dimensional information (Mayasari et al., 2022). The most straightforward, quick, and efficient way to examine the chemical profile of plant extracts is TLC, as opposed to other methods (Hawrył et al., 2016; Mayasari et al., 2022; Zahiruddin et al., 2021). Additionally, the TLC method can be utilized for qualitative or quantitative separations and is a cost-effective, easy, and universal separation approach that can be applied in any laboratory (Braz et al., 2012). TLC can be used for plant material quality control, such as fingerprint profiles to determine the chemical content of an extract, as well as quantitative examination of markers in specific high-quality plant medicines (Braz et al., 2012; Rafi et al., 2023). The following herbs have been identified and authenticated by TLC fingerprint analysis: *Curcuma longa* L. (Kartini et al., 2021), *Orthosiphon stamineus* (Kartini et al., 2020), *Curcuma xanthorrhiza* (Kartini et al., 2023), *Melastoma malabathricum* (Mayasari et al., 2022), and *Sida rhombifolia* (Rafi et al., 2023). TLC fingerprints can accurately authenticate and identify plant materials. As a result, obtaining trustworthy TLC fingerprints that represent the plant materials' bioactive chemicals and chemically unique components is critical (Zahiruddin et al., 2021)

Since it was first used for main qualitative profiles, the TLC method has quickly grown into a multi-target quantitative analysis tool that has many uses in the field of plant medicine (Liu et al., 2018). For the purpose of assessing the adulteration and purity of traditional medicinal constituents (Zahiruddin et al., 2021), plant authentication (Mutiah et al., 2019), and quality control of medicinal raw materials derived from plants with similar chemical profiles, this technology is utilized (Kartini et al., 2023). This makes this method appropriate for identifying the distinct chemical profile attributes of various plant species or even the same plant with spatial variations in its cultivation. By employing TLC densitometry and quantitative analysis of Peak area and Rf scanned with 254 nm and 366 nm UV lamps, this study utilized metabolic fingerprinting to determine whether variations in the metabolite profile of *P. canescens* Jack were attributable to geographical variations in the growing location. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to evaluate the data, which had been collected as an Excel data sheet. This study is the first to examine the metabolites of *P. canescens* Jack leaves from Muara Labuh (West Sumatra), Batang Asai (Jambi), Rejang Lebong (South Curup), Taman Hutan Raya Rajo Lelo (Central Bengkulu), Taba Penanjung (Central Bengkulu), and Empat Lawang (South Sumatra).

P. canescens Jack is cultivated in 10 regions with varied geographical conditions. This study was conducted on three distinct islands—Sumatra, Java, and Kalimantan—in Indonesia, an archipelagic nation. The sampling locations were based on altitude differences and sample availability in the area. The locations from which it was extracted in Sumatra are as follows: Muara Labuh in West Sumatera, Batang Asai in Jambi, Rejang Lebong in Curup, Taman Hutan Raya Rajo Lelo in Central Bengkulu, Taba Penanjung in Central Bengkulu, and Empat Lawang in South Sumatera. In Java, the samples were collected from the areas of Bogor (West Java), Pangandaran (West Java), and Banyumas (Central Java). While in Kalimantan, they were collected from Bangelan (South Kalimantan). Subsequently, the leaves of three specific trees were harvested from each location. Botanist Dr. Djoko Santosa verified the validity of each sample.

This study aims to investigate the impact of geographical variations on the leaf variations of *P. canescens* Jack, a plant that exhibits wide distribution across diverse regions and altitudes in Indonesia. The environmental conditions in each region will have an impact on the profile of secondary metabolites (Pranatami et al., 2023). Plants' chemical reactions and responses will vary in response to environmental variations, thereby influencing the production of metabolites. Additionally, plant maturity can impact the concentration of a secondary metabolite. The quantity of secondary metabolites exhibits a positive correlation with the age of the plant (L. Yang et al., 2018). Overall, the purpose of this study is to determine whether the metabolite profiles of *P. canescens* Jack leaves from various growing areas are comparable or dissimilar.

MATERIALS AND METHODS

Materials

Sungkai leaves (*Peronema canescens* Jack), 20×20 cm silica gel plate 60 F_{254} (Merck), 96% ethanol, methanol, n-hexane (Merck, Darmstadt, Germany), and ethyl acetate (Smart Lab, Smart Lab Indonesia).

Equipment

The CAMAG TLC Scanner 3 and D2 and W lamps were employed to observe TLC plates under UV light at 254 nm and 366 nm. The Rf values and area under the curve were calculated by the WinSTATS software. Chemometric analysis was performed by the Metaboanalyst 06 software.

Methods

Plant Material

The sungkai leaves were collected from ten locations: Bogor (BGA, BGB, BGC) (6°32'08"S 106°37'44"E), Pangandaran (PDA, PDB, PDC) (7°29'22"S 108°23'25"E), Banyumas (BMA, BMB, BMC) (7°35'30"S 109°18'40"E), South Kalimantan (KSA, KSB, KSC) (2°48'21"S 115°15'40"E), Rejang Lebong (RLA, RLB, RLC) (South Curup) (3°30'22"S 102°31'03"E), Batang Asai (BAA, BAB, BAC) (Jambi) (2°26'37"S 102°19'17"E), Muaro Labuh (MLA, MLB, MLC) (West Sumatra) (1°28'12"S 101°04'03"E), Taba Penanjung (TPA, TPB, TPC) (Central Bengkulu) (3°42'38"S 102°27'31"E), Empat Lawang (ELA, ELB, ELC) (South Sumatra) (3°37'22"S 103°02'56"E), and Taman Hutan Raya Rajo Lelo (Central Bengkulu) (THA, THB, THC) (3°44'14"S 102°19'01"E). These locations have altitudes ranging from 10 to 700 metres above sea level. This region offers diverse conditions for the distribution of *P. canescens* Jack plants, including variations in elevation, temperature, and precipitation.

Three leaves from three trees situated in distinct locations were collected from each area. The leaves were collected from sungkai trees with a tree circumference ranging from 35 cm to 90 cm. The leaves were recognized in Unit II of the Department of Pharmaceutical Biology at the Faculty of Pharmacy at Universitas Gadjah Mada, with the ID number 35.21.11/UN1/FFA.2/BF/PT/2022. The leaves of *P. canescens* Jack was dried at 40ºC in a drying cabinet for three days, pulverized, and sieved through a 40-mesh sieve before extraction.

Preparation for Extraction

20 grams of simplicia was measured by an analytical balance with a sensitivity of 0.001 milligrams. The sample was then extracted with 100 milliliters of ethanol by the maceration method at a temperature ranging from 25 to 30 degrees Celsius for a duration of 24 hours. The filtrate was evaporated by a rotary evaporator. The ethanol extract was dissolved in 1 ml of ethanol and underwent filtration by a 0.45 µm membrane filter, rendering it and prepared for analysis. The *P. canescens* Jack leaf extract was subjected to metabolite analysis by the densitometry TLC method (Zahiruddin et al., 2021).

TLC Densitometry

The extract samples were analyzed by thin-layer chromatography densitometry. A silica gel 60 F_{254} plate measuring 20x10 cm was used for the analysis. The sample spotting procedure was conducted with a CAMAG Linomat 5, with a spacing of 1 cm between spots and a volume of 5 µL for each spot. Once all the samples were placed on the plate, the plate was immersed in a vessel that had been soaked with the mobile phase, specifically n-hexane: ethyl acetate (14:6, v/v), and allowed to develop. The plate that was created underwent scanning under 254 nm and 366 nm UV light by a CAMAG TLC Scanner 3. The obtained data was subsequently analyzed by WinCATS Planar Chromatography Manager software. Each sample underwent three replications to ensure accurate results (Mayasari et al., 2022) (Rafi et al., 2023).

Chemometric analysis

The compounds identified by thin-layer chromatography (TLC) in each extract were further analyzed to compare their metabolite profiles. The data was normalized by the presence (area of chemicals found on the TLC plate)

or absence (Value 0) of compounds in distinct extracts. The data for the peak area of each Rf value was inputted into Microsoft Excel. The Excel data underwent multivariate principal component analysis (PCA) and Hierarchical Clustering Analysis (HCA) "Dendrogram and Heatmap". The normalized data were then processed by Metaboanalyst 06 software. The collected data were expressed as principal component (PC) values, which characterize the similarities and differences in the chemical composition of plant extracts from different places. The HCA clustering *P. canescens* Jack was based on a similarity chemotype (Zahiruddin et al., 2021).

RESULTS AND DISCUSSIONS

The prepared samples were evaluated by TLC densitometry. The TLC analysis performed to ascertain the overall chemical profile was non-targeted. In the first stage, the separation resolution of chemical components in a single process was optimized by optimizing the TLC conditions. The selected mobile phase n-hexane: Ethyl acetate (7:3) was utilized to analyze extracts from various regions on a stationary phase silica gel F_{254}. Varying numbers of spots were observed in each area as a consequence of scanning with UV lamps operating at 254 nm and 366 nm. This finding demonstrates that chemical constituents in plants exhibit variations. TLC metabolite fingerprinting was performed by examining the chromatogram results, as shown in Figure 1. In the scan result on the 254 nm lamp, the maximum peak areas were observed at Rf 0.36, which were also present in nearly all samples. The peak areas for all samples were 0.03, 0.09, 0.36, 0.63, 0.85, and 0.95. Different quantities were indicated by Rf 0.25 and 0.63. Based on the result in Figure 1, the bands were shown more in plates under 366 nm compared with 254 nm. Many spots could be detected in the 366 nm but did not appear in the 254 nm (the compounds did not have chromophores).

Figure 2 (a)-1(b)-1(d) showed several peaks at Rf 0.00–0.36, varying in number, and no discernible major peaks at Rf 0.4–0.8. There were peaks in Figures 2 (c) and 1(d) that varied from Rf 0.4-0.95. Variations in secondary metabolites generated as a result of geographical conditions account for the peaks that appear in specific regional sample tracks. Each peak's variations and similarities reveal the distinct chemical composition profile of each location. The scan result in TLC 366 nm found different peak areas in Rf 0.6-0.9. Figure 3 (c), (d), and (e) in Rf 0.6 and 0.9 had significant peak areas, and these were the markers for discriminating *P. canescens* Jack in geographical variation. The Empat Lawang and Batang Asai in Figure 3 (c) show that the Rf 0.6 peak area of Batang Asai was higher than that of Empat Lawang. Meanwhile, in Figure 3 (d), Pangandaran and Kabupaten Bogor had similar peaks in Rf 0.6 and 0.9, while Kalimantan Selatan and Banyumas had different paths.

The process of metabolite fingerprinting via TLC involves the analysis of photodensitogram outcomes, where the peaks observed on individual sample tracks are utilized to determine the similarities and differences. In order to observe distinct spot separation, TLC results must possess optimal resolution. Based on the peak area that appears at a particular Rf on each track, the distinctions between each sample can be observed in this study. Implementing this straightforward technique enables the discrimination of particular chemical constituents among samples sourced from various geographical areas (Kowalska & Sajewicz, 2022). In addition to determining the precise constituents of this plant, multivariate chemometric analysis was applied to the TLC results in order to calculate comparisons and contrasts between *P. canescens* Jack from different geographic regions. The chemical composition of samples obtained from various geographical locations will be clustered by chemometric results. Moreover, the other method has been carried out for comparison of metabolite by FTIR on kimchi (Kim & Ha, 2024), *Zingiber officinale* and *Piper longum* (Sahoo & Umashankara, 2022), and Curcuma species with the ^{1}H-NMR method (Nurani et al., 2021). It is the first metabolite profiling of *P. canescens* Jack by TLC fingerprinting.

Historically, the leaves have been employed for their supposed antimalarial and fever-reducing properties, and postpartum remedies. The chemical compound of *Peronema canescens* Jack leaves is the clerodane diterpenoid compound, which is associated with its immune-enhancing and antimalarial properties. The efficacy as a postnatal medication can be attributed to its flavonoid content, which exhibits antipyretic and antibacterial effects. Furthermore, clerodane, diterpenoids, and alkaloid compounds present in leaves are identified as being responsible for their antimalarial properties (W. Yang et al., 2020).

The TLC densitometry data were converted to Rf values for each sample, after which the peak area data were analyzed. Chemometric analysis was performed on the data by Principal Component Analysis (PCA) and

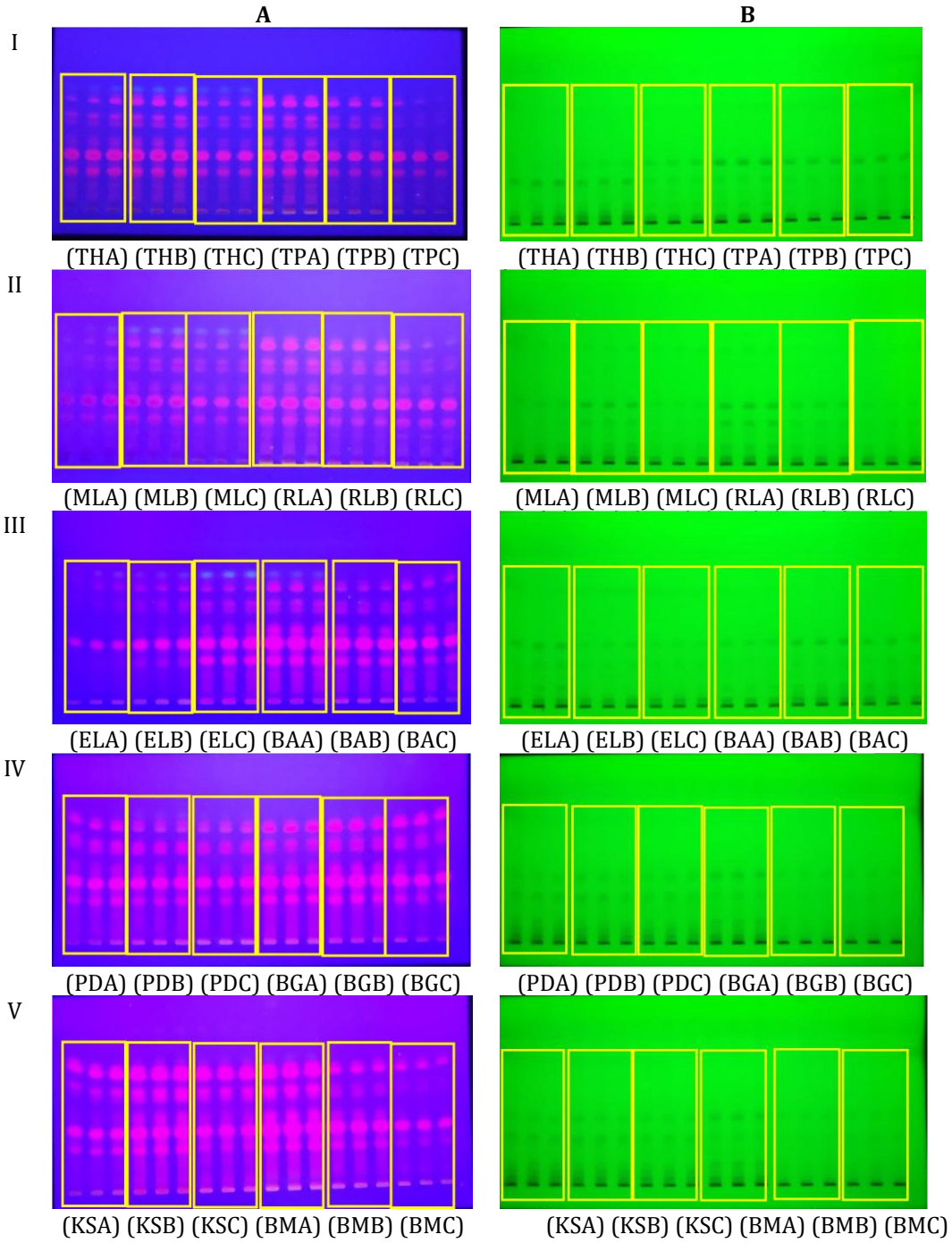


Figure 1. TLC Fingerprinting of *P.canescens* Jack From (a) Taman Hutan Raya (A, B, C) and Taba Penanjung (A, B, C), (b) Muara Labuh (A, B, C), and Rejang Lebong (A, B, C), (c) Empat Lawang (A, B, C), and Batang Asai (A, B, C), (d) Pangandaran (A, B, C), and Bogor (A, B, C), (e) Kalimantan Selatan (A, B, C), and Banyumas (A, B, C), A= 366 nm, B= 254nm, Mobile Phase: n-Hexane: Etyl Acetate (7:3)

Hierarchical Clustering Analysis (HCA). The Principal Component Analysis (PCA) can easily show patterns within a data set and differences between sets of data, and it is subsequently employed to reduce the original data while preserving the original variation (Xiao et al., 2022). Principal Component Analysis (PCA) characterizes the degree of similarity between samples; the closer the PC value, the more similar the samples. The contribution of PC1 and PC2 is depicted in the score plot in Figure 4 (a), which was obtained from the analysis conducted by Metaboanalyst version 6.0. PC1 and PC2 each account for 32.6% and 41.3%, respectively, and a total of 73.9%.

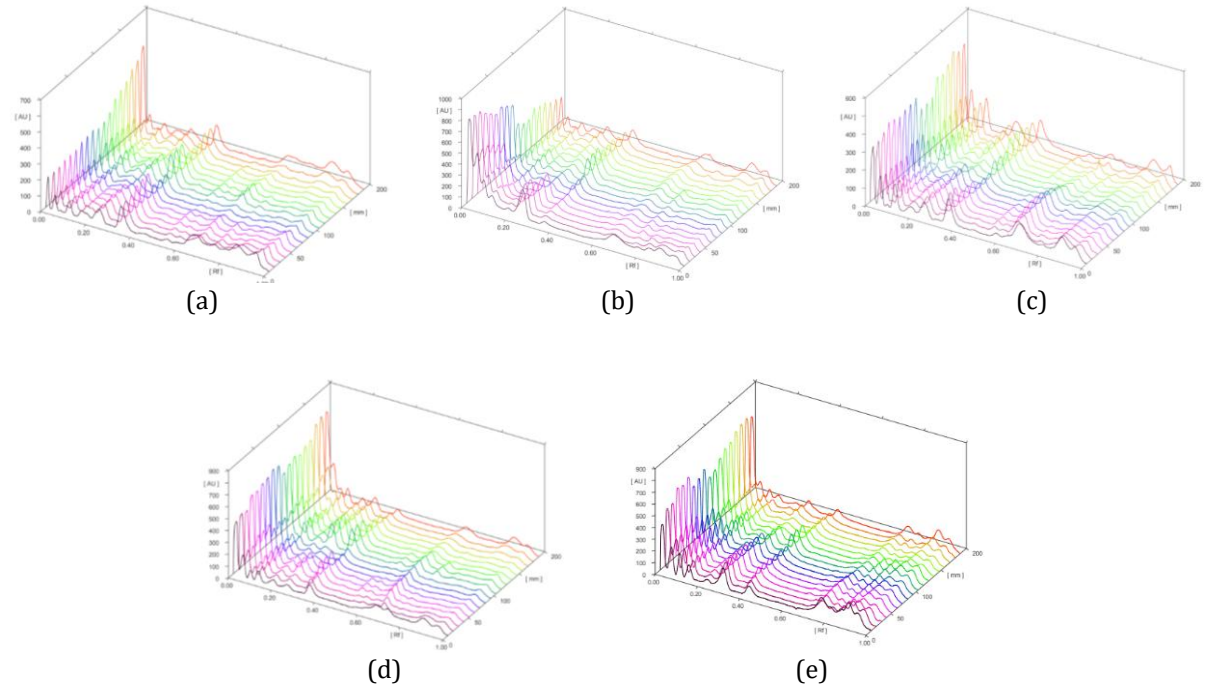


Figure 2. Photodensitogram of a fluorescent TLC Fingerprinting plate using a densitometer and the software WINCATS at a wavelength of 254 nm (a). Samples TH= Taman Hutan Raya, TP= Taba Penanjung, (b) ML= Muara Labuh, RL= Rejang Lebong, (c) EL= Empat Lawang, BA= Batang Asai, (d) PD= Pangandaran, BG= Bogor (e) KS= South Kalimantan, BM= Banyumas

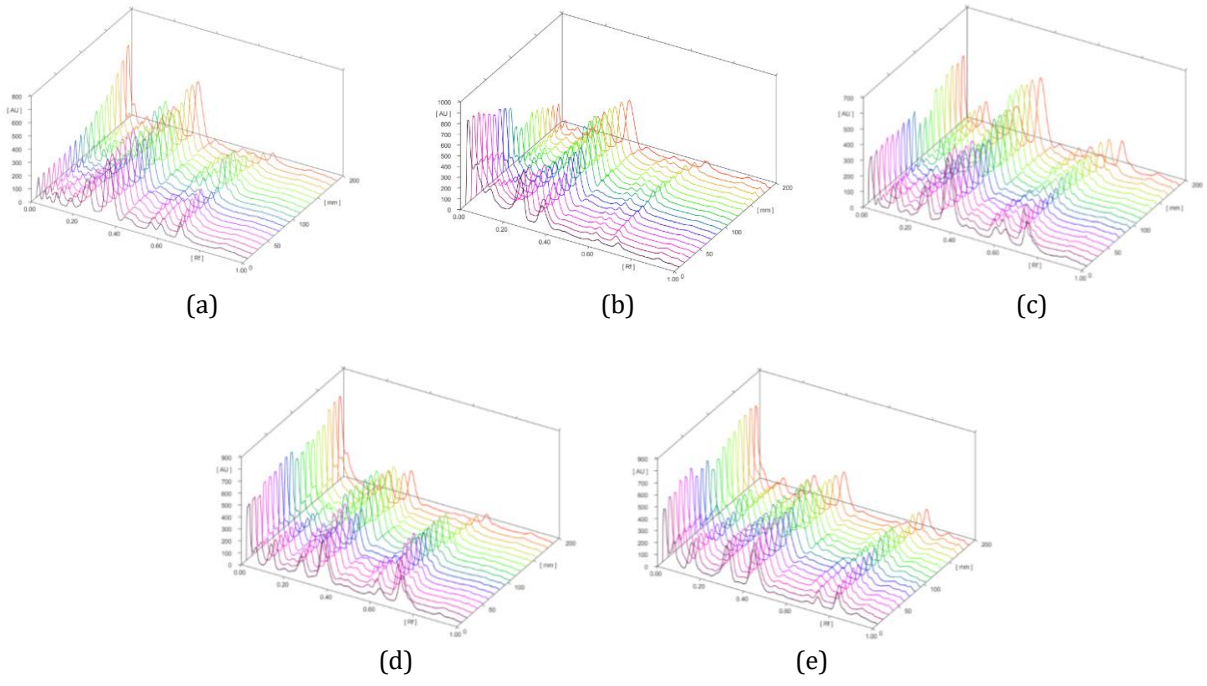
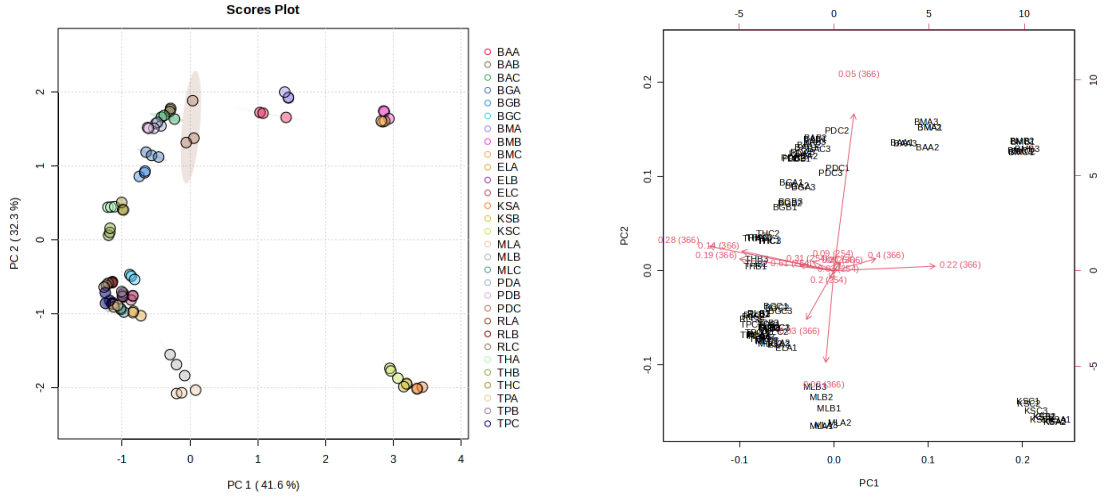


Figure 3. Photodensitogram of a fluorescent TLC fingerprint plate using a densitometer and the software WINCATS at a wavelength of 366 nm (a). Samples TH= Grand Forest Park, TP= Taba Penanjung, (b) ML= Muara Labuh, RL= Rejang Lebong, (c) EL= Empat Lawang, BA= Batang Asai, (d) PD= Pangandaran, BG= Bogor (e) KS= South Kalimantan, BM= Banyumas



(a) (b)

Figure 4. (a) Score Plot of PCA Analysis Results, (b) Biplot of PCA Analysis Results Using the Rf Variable with a Certain Peak Area

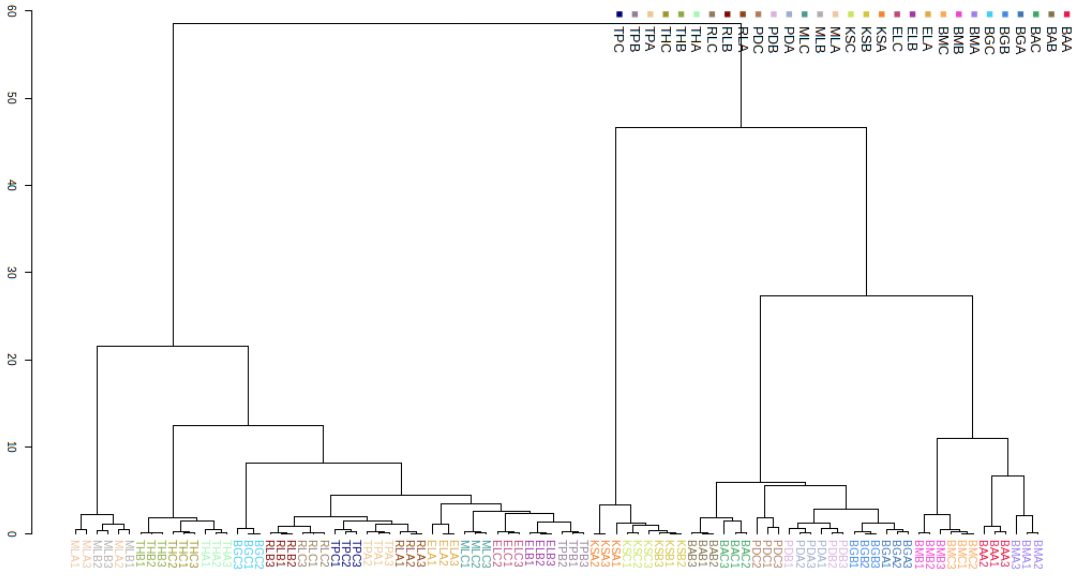
The PCA results demonstrate that the chemical composition of each growing area is comparable, suggesting that the regions are grouped according to this similarity. The leaves grown on *P. canescens* Jack in Sumatra exhibited a similar chemical profile, forming one cluster.

The biplot in Figure 4 (b) indicates that the variable PC1 is most substantially influenced by the peak area at Rf 0.22 (366 nm). Rf value of 0.22 at a wavelength of 366 nm can be a distinguishing factor for *P. canescens* Jack, which comes from the South Kalimantan and Banyumas (Central Java) regions. Rf 0.08 (366 nm) has a significant impact on PC2 due to its higher value on PC2, making this variable distinctive in *P. canescens* Jack leaves originating from Muara Labuh (West Sumatra). Rf 0.28 (366 nm) shows a significant impact on PC2; it's the unique compound *P. canescens* Jack from Taman Hutan Raya (Central Bengkulu).

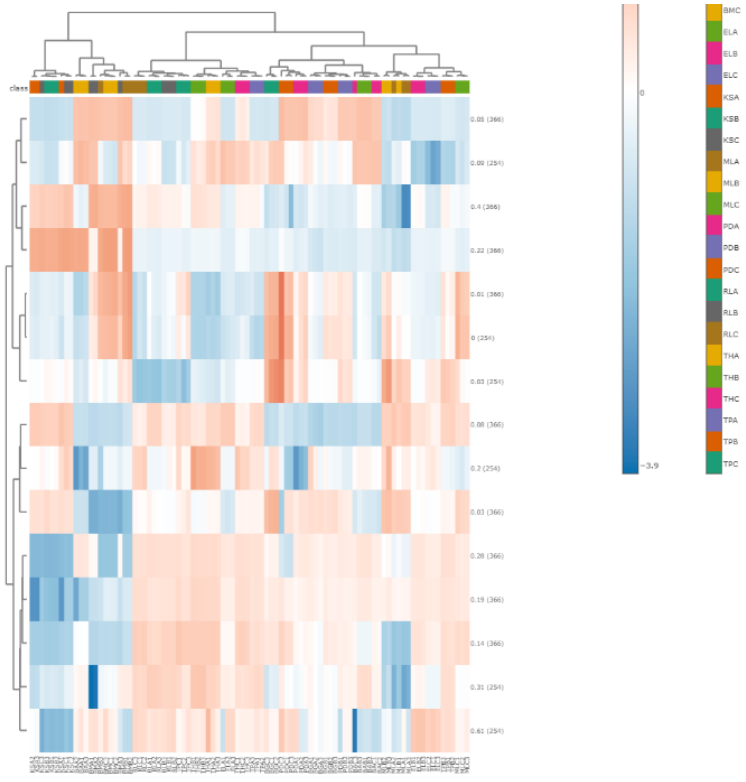
The results indicate that HCA clusters *P. canescens* Jack leaves according to the similarity of their metabolite profiles into four clusters (Figure 5). The heatmap helps find variables that appear to be characteristic of each sample cluster. The metabolite profiles of *P. canescens* Jack leaves from Taba Penanjung, Rejang Lebong, Empat Lawang, Taman Hutan Raya, and Muara Labuh are found to be identical (Cluster 1). Batang Asai, Pangandaran, and Bogor (Cluster 3) indicate a close relationship, although there is one tree from the Bogor area that is included in the Sumatra cluster. On the other hand, samples from South Kalimantan (Cluster 2) and Banyumas (4) each form distinct clusters, suggesting a significant in chemical content. This phenomenon may be attributed to the proximity of these geographical places.

The geographical coordinates of latitude, longitude, and altitude exert an influence on the duration of daylight, temperature, and precipitation, hence indirectly impacting the accumulation of secondary metabolites in plants. According to research by Vilkickyte et al. (2021) and Sri Sulasmi et al. (2019), this investigation obtained the optimal secondary metabolites, including polyphenol content, in this plant. The environmental change was better to avoid reduced annual temperatures or sunshine duration and colder air temperatures at higher elevations. Furthermore, the altitude of the planting area did not significantly affect the difference in polyphenols. These findings confirm the conclusion that variations in altitude did not result in significant differences in the metabolite profile. Climate also affects the production of secondary metabolites, and most terpenes will increase their synthesis in hot environments. Meanwhile, phenolic compounds will increase their production in cold environments (Qaderi et al., 2023).

Geographic factors have a significant impact on the diversity of secondary metabolites. Metal content, soil type, and pH all have a substantial impact on the secondary metabolite production of *Zataria multiflora* Boiss (L. Yang et al., 2018). The phenol and flavonoid content of *Vaccinium Vitis-idea* L. leaves is positively correlated with air humidity, longitude, and altitude of the collection site, as well as soil macronutrients (Karimi et al., 2020). Plants that are native to highlands, semi-



(A)



(B)

Figure 5. Dendogram (A) and HeatMap Hierarchical Clustering Analysis (B)

arid regions, or cold climates have the highest concentration of phenolic compounds (Kabtni et al., 2020). There was a positive correlation observed between the height of the growing area and the concentrations of flavonoids, phenols, and caffeic acid. Conversely, the sesquiterpene lactone of *Arnica montana* exhibited an inverse correlation with the aforementioned parameter (Zidorn, 2010).

Based on the findings of this study, TLC fingerprints can be used to show the similarities and differences in *P. canescens* Jack metabolite

between each location based on the Rf value, and the results are classified into four groups. This shows how TLC fingerprints can be combined with chemometrics to confirm the identity of plant species through the similarities between plant extracts and fingerprint characteristics. The study's obvious benefit is that the TLC analysis method is quite inexpensive to use, and that basic sample processing can yield important metabolite profile data.

CONCLUSION

The climatic and physiological conditions and geographic location play an important role in the phytochemical properties of *P. canescens* Jack leaves. The findings of our study provide compelling evidence in support of TLC. Densitometry can serve as a convenient method for assessing plant metabolite profiles. The results of TLC can be effectively processed and evaluated by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The Principal Component Analysis (PCA) yielded PC1 values of 41.3% and PC2 values of 23.6%, which indicate the extent of sample variations. Additionally, the Hierarchical Cluster Analysis (HCA) successfully classified the samples into four distinct clusters. The results also revealed that locations in close proximity will have very similar environments. The macronutrient conditions, soil quality, light, temperature, and humidity have an impact on the phenolic and terpenoid chemical content of *P. canescens* Jack leaves. The changes in plant metabolite profiles cannot be attributed to a single component but rather to a variety of complicated biotic and abiotic factors.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in the manuscript

REFERENCES

Braz, R., Wolf, L. G., Lopes, G. C., & De Mello, J. C. P. (2012). Quality control and TLC profi le data on selected plant species commonly found in the Brazilian market. *Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy*, *22*(5), 1111–1118. https://doi.org/10.1590/S0102

Carvalho, F. V., Fonseca Santana, L., Diogenes A. da Silva, V., Costa, S. L., Zambotti-Villelae, L., Colepicolo, P., Ferraz, C. G., & Ribeiro, P. R. (2021). Combination of a multiplatform metabolite profiling approach and chemometrics as a powerful strategy to identify bioactive metabolites in Lepidium meyenii (Peruvian maca). *Food Chemistry*, *364*. https://doi.org/10.1016/j.foodchem.2021.130453

Demasi, S., Caser, M., Lonati, M., Cioni, P. L., Pistelli, L., Najar, B., & Scariot, V. (2018). Latitude and altitude influence secondary metabolite production in peripheral alpine populations of the mediterranean species lavandula angustifolia mill. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.00983

Dillasamola, D., Aldi, Y., Wahyuni, F. S., Rita, R. S., Dachriyanus, Umar, S., & Rivai, H. (2021). Study of Sungkai (Peronema canescens, Jack) leaf extract activity as an immunostimulators with in vivo and in vitro methods. *Pharmacognosy Journal*, *13*(6), 1397–1407. https://doi.org/10.5530/PJ.2021.13.177

Hawrył, A., Ziobro, A., Świeboda, R., Hawrył, M., & Waksmundzka-Hajnos, M. (2016). TLC Profiles of Selected Cirsium Species with Chemometrics in Construction of Their Fingerprints. *Journal of Chromatographic Science*, *54*(7), 1096–1104. https://doi.org/10.1093/chromsci/bmw064

Kabtni, S., Sdouga, D., Bettaib Rebey, I., Save, M., Trifi-Farah, N., Fauconnier, M. L., & Marghali, S. (2020). Influence of climate variation on phenolic composition and antioxidant capacity of Medicago minima populations. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-65160-4

Karimi, A., Krähmer, A., Herwig, N., Schulz, H., Hadian, J., & Meiners, T. (2020). Variation of Secondary Metabolite Profile of Zataria multiflora Boiss. Populations Linked to Geographic, Climatic, and Edaphic Factors. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00969

Kartini, K., Andriani, Y. A., Priambodo, W., Jayani, N. I. E., & Hadiyat, M. A. (2021). Validating and developing TLC-based fingerprinting for Curcuma longa L. © *2021 Journal of Pharmacy & Pharmacognosy Research*, *9*(5), 704–715. http://jppres.com/jppresOriginalArticle

Kartini, K., Dewi, E. R., Achmad, F., Jayani, N., Hadiyat, M. A., & Avanti, C. (2020). Thin

Layer Chromatography Fingerprinting and Clustering of Orthosiphon stamineus Benth. from Different Origins. *Phcogj.Com Pharmacognosy Journal*, *12*(1), 1683–1691. https://doi.org/10.5530/pj.2020.12.257

Kartini, K., Sabatini, S. S., Haridsa, N. M., Jayani, N. I. E., Setiawan, F., & Hadiyat, M. A. (2023). TLC-fingerprinting and chemometrics for identification of Curcuma xanthorrhiza from different geographical origins in Indonesia. *Biodiversitas*, *24*(12), 6557–6566. https://doi.org/10.13057/biodiv/d241217

Kim, S. Y., & Ha, J. H. (2024). Rapid determination of the geographical origin of kimchi by Fourier transform near-infrared spectroscopy coupled with chemometric techniques. *Scientific Reports*, *14*(1), 24581. https://doi.org/10.1038/s41598-024-74662-4

Klau, M. E., Rohaeti, E., Rafi, M., Artika, I. M., Ambarsari, L., & Nurcholis, W. (2023). Metabolite Profiling of Curcuma zanthorrhiza Varieties Grown in Different Regions Using UHPLC-Q-Orbitrap-HRMS and Chemometrics Analysis. *Biointerface Research in Applied Chemistry*, *13*(1). https://doi.org/10.33263/BRIAC131.026

Kowalska, T., & Sajewicz, M. (2022). Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications. In *Molecules* (Vol. 27, Issue 19). MDPI. https://doi.org/10.3390/molecules27196607

Latief, M., Anggun, ;, Fisesa, T., Putri, ;, Sari, M., Indra, ;, & Tarigan, L. (2021). Jurnal Farmasi Sains dan Praktis AKTIVITAS ANTIINFLAMASI EKSTRAK ETANOL DAUN SUNGKAI (PERONEMA CANESCENS JACK) PADA MENCIT TERINDUKSI KARAGENAN ANTI-INFLAMMATORY ACTIVITY OF SUNGKAI LEAVES (PERONEMA CANESCENS JACK) ETHANOL EXTRACT IN CARRAGEENAN INDUCED MICE. In *JFSP* (Vol. 7, Issue 2). http://journal.ummgl.ac.id/index.php/pharmacy

Latief, M., Sari, P. M., Fatwa, L. T., Tarigan, I. L., & Rupasinghe, H. P. V. (2021). Antidiabetic Activity of Sungkai (Peronema canescens Jack) Leaves Ethanol Extract on the Male Mice Induced Alloxan Monohydrate. *Pharmacology and Clinical Pharmacy Research*, *6*. https://doi.org/10.15416/pcpr.v4i3.31666

Liu, X., Ahlgren, S., Korthout, H. A. A. J., Salomé-Abarca, L. F., Bayona, L. M., Verpoorte, R., & Choi, Y. H. (2018). Broad range chemical profiling of natural deep eutectic solvent extracts using a high performance thin layer chromatography–based method. *Journal of Chromatography A*, *1532*, 198–207. https://doi.org/10.1016/j.chroma.2017.12.009

Mayasari, D., Murti, Y. B., Pratiwi, S. U. T., Sudarsono, S., Hanna, G., & Hamann, M. T. (2022). TLC-Based Fingerprinting Analysis of the Geographical Variation of Melastoma malabathricum in Inland and Archipelago Regions: A Rapid and Easy-to-Use Tool for Field Metabolomics Studies. *Journal of Natural Products*, *85*(1), 292–300. https://doi.org/10.1021/acs.jnatprod.1c00622

Muharni, muharni, Ferlinahayati, F., Yohandini, H., Riyanti, F., & Pakpahan, N. A. P. (2021). The THE ANTICHOLESTEROL ACTIVITY OF BETULINIC ACID AND STIGMASTEROL ISOLATED FROM THE LEAVES OF SUNGKAI (PARONEMA CANESCENS JACK). *International Journal of Applied Pharmaceutics*, *13*(2), 198–203. https://doi.org/10.22159/ijap.2021v13i2.40372

Mutiah, R., Hadya, C. M., Burhan Ma'arif, Z. A., Bhagawan, W. S., Annisa, R., Indrawijaya, Y. Y. A., Huwaida, F. I., Ria Ramadhani, D. A., Susilowati, R., & Taufik, I. (2019). Metabolite fingerprintiing of eleutherine palmifolia (L.) merr. By hptlc-densitometry and its correlation with anticancer activities and in Vitro Toxicity. *Indonesian Journal of Pharmacy*, *30*(3), 157–166. https://doi.org/10.14499/indonesianjpharm30iss3pp157

Nagana Gowda, G. A., & Raftery, D. (2023). Quantitative NMR Methods in Metabolomics. In *Handbook of Experimental Pharmacology* (Vol. 277, pp. 143–164). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/164_2022_612

Nurani, L. H., Rohman, A., Windarsih, A., Guntarti, A., Riswanto, F. D. O., Lukitaningsih, E., Fadzillah, N. A., & Rafi, M. (2021). Metabolite fingerprinting using1h-nmr spectroscopy and chemometrics for classification of three curcuma species from different origins. *Molecules*, *26*(24). https://doi.org/10.3390/molecules26247626

Plumb, R. S., Gethings, L. A., Rainville, P. D., Isaac, G., Trengove, R., King, A. M., & Wilson, I. D. (2023). Advances in high throughput LC/MS

based metabolomics: A review. In *TrAC - Trends in Analytical Chemistry* (Vol. 160). Elsevier B.V. https://doi.org/10.1016/j.trac.2023.116954

Pranatami, D. A., Atiqah, N., Mariska, R., & Walisongo, S. J. (2023). *The Differences in Adaptation Between Lowland and Highland Populations*. *5*.

Putranto, A. M. H. (2014). EXAMINATION OF THE SUNGKAI'S YOUNG LEAF EXTRACT (Peronema canescens) AS AN ANTIPIRETIC, IMMUNITY, ANTIPLASMODIUM AND TERATOGENITY IN MICE (Mus.muculus). *International Journal of Science and Engineering*, *7*(1). https://doi.org/10.12777/ijse.7.1.30-34

Qaderi, M. M., Martel, A. B., & Strugnell, C. A. (2023). Environmental Factors Regulate Plant Secondary Metabolites. In *Plants* (Vol. 12, Issue 3). MDPI. https://doi.org/10.3390/plants12030447

Rafi, M., Yolanda, S. R., Septaningsih, D. A., Bintang, M., Aminah, N. S., Insanu, M., & Rohman, A. (2023). Identification of Sida rhombifolia from Its Related Plants Using Thin-Layer Chromatographic Analysis. *Indonesian Journal of Chemistry*, *23*(1), 21–32. https://doi.org/10.22146/ijc.73077

Rohaeti, E., Karunina, F., & Rafi, M. (2021). Ftir-based fingerprinting and chemometrics for rapid investigation of antioxidant activity from syzygium polyanthum extracts. *Indonesian Journal of Chemistry*, *21*(1), 128–136. https://doi.org/10.22146/ijc.54577

Sahoo, M. R., & Umashankara, M. S. (2022). FTIR Based Metabolomics Profiling and Fingerprinting of Some Medicinal Plants: An Attempt to Develop an Approach for Quality Control and Standardization of Herbal Materials. *Pharmacognosy Research*, *15*(1), 163–167. https://doi.org/10.5530/097484900288

Salomé-Abarca, L. F., Van Den Hondel, C. A. M. J. J., Erol, Ö., Klinkhamer, P. G. L., Kim, H. K., & Choi, Y. H. (2021). HPTLC-based chemical profiling: An approach to monitor plant metabolic expansion caused by fungal endophytes. *Metabolites*, *11*(3). https://doi.org/10.3390/metabo11030174

Tarigan, I. L., Sutrisno, Rumaida, Aini, I. P. S., & Latief, M. (2022). Isolation of a Flavone Apigenin and a Steroids Squalene from Peronema canescens Jack Leaves with Anti-Inflammatory Activities. *Pharmacognosy Journal*, *14*(6), 744–752. https://doi.org/10.5530/pj.2022.14.162

Verma, N., & Shukla, S. (2015). Impact of various factors responsible for fluctuation in plant secondary metabolites. In *Journal of Applied Research on Medicinal and Aromatic Plants* (Vol. 2, Issue 4, pp. 105–113). Elsevier GmbH. https://doi.org/10.1016/j.jarmap.2015.09.002

WHO. (2000). *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*.

Xiao, Q., Mu, X., Liu, J., Li, B., Liu, H., Zhang, B., & Xiao, P. (2022). Plant metabolomics: a new strategy and tool for quality evaluation of Chinese medicinal materials. In *Chinese Medicine (United Kingdom)* (Vol. 17, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s13020-022-00601-y

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. In *Molecules* (Vol. 23, Issue 4). MDPI AG. https://doi.org/10.3390/molecules23040762

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in Pharmacological Activities of Terpenoids. In *Natural Product Communications* (Vol. 15, Issue 3).

Yunita, O., Rantam, A., & Yuwono, M. (2019). *Metabolic fingerprinting of Sauropus androgynus (L.) Merr. leaf extracts*. https://doi.org/10.29090/psa.2019.01.017.0043

Zahiruddin, S., Parveen, A., Khan, W., Parveen, R., & Ahmad, S. (2021). TLC-Based Metabolite Profiling and Bioactivity-Based Scientific Validation for Use of Water Extracts in AYUSH Formulations. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/10.1155/2021/2847440

Zidorn, C. (2010). *Altitudinal variation of phenolics contents in flowering heads of the Asteraceae family*. https://www.researchgate.net/publication/216674608