Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2024; 10 (2): 257 – 269
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2024.v10.i2.16534

Metabolite Fingerprints and Chemometrics-Based Approach for Discrimination of Miana (*Coleus scutellarioides*) Variety: Authentication of Traditional Medicine Raw Materials

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Article Info:
Received: 05 September 2023
in revised form: 22 April 2024
Accepted: 01 September 2024
Available Online: 01 October 2024

Keywords:
Multivariate analysis
Coleus scutellarioides
FT-IR
Metabolomics spectroscopy

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ABSTRACT

Background: Miana (*Coleus scutellarioides*) is a plant from the Lamiaceae family consisting of several varieties that are traditionally used as medicinal materials, especially its leaves. **Objectives:** The study aims to classify/discriminate miana varieties based on FT-IR spectroscopic profiles and chemometric analysis (PCA, HCA, and PLS). Material and Methods: There are four samples used in this study, namely purple miana, green miana, batik miana, and combination miana (combination colour). The powders of the four miana samples were analyzed using FT-IR spectroscopy, then analyzed by chemometric techniques using PCA, HCA, and PLS to see the clustering patterns and functional group markers of the samples. **Results:** Based on chemometric analysis of FT-IR data, the four species of miana leaves showed grouping based on their varieties, respectively. The total PC value was 99.5% (PC-1 97.7% and PC-2 1.8%). HCA analysis at a distance of 2.5 resulted in 4 groups: a (DMB, DMK, and DMU), b (DMU and DMK), c (DMU and DMK), and d (DMH). PLS analysis using VIP scores showed C–H and C=O groups with values > 1. **Conclusions:** The combination of FT-IR and chemometrics can be applied to discriminate miana samples in quality control and authentication of traditional medicine raw materials. Analysis with LC-MS/MS and NMR instruments is needed for further analysis and support compounds that have the potential to distinguish the four miana varieties.

How to cite (APA 6th Style):
Syahruni, R., Umar, A. H., Lido, F. (2024). Metabolite Fingerprints and Chemometrics-Based Approach for Discrimination of Miana (*Coleus scutellarioides*) Variety: Authentication of Traditional Medicine Raw Materials. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 10*(2), 257-269. doi:10.22487/j24428744.2024.v10.i2.16534

INTRODUCTION

The Miana plant (*Coleus scutellarioides* (L.) Benth.) is a family of Lamiaceae and is one of the ornamental plants consisting of various varieties that are famous for their attractive leaf colours and shapes and diverse types. Traditionally, the leaves of this plant are used as a medicinal substance (Silalahi et al., 2015; Bismelah et al., 2022; Akaputra et al., 2023; Tambaru et al., 2023). Miana leaves contain essential oils, tannins, flavonoids, eugenol, steroids, tannins, saponins, phytol, rosmanic acid, and quercetin (Dörr et al., 2019; Aziz et al., 2021; Bismelah et al., 2022; Akaputra et al., 2023). Generally, miana leaves in fresh form (juice, brew, and decoction) are used to treat asthma, cough, menstrual cycle, neutralise toxins, increase appetite, diarrhoea, and worms (Silalahi et al., 2015; Bismelah et al., 2022; Tambaru et al., 2023).

Research related to the identification and activity testing of several types of miana leaves, such as red miana, purple miana, and green miana has been widely explored (Ayu et al., 2018; Bismelah et al., 2022; Akaputra et al., 2023). Differences in the colour of miana leaves indicate differences in the compounds contained in the plant. Despite the striking color difference, it is still very difficult to distinguish the type of miana, if it is substituted in the form of dry or powder simplicia. If this mixing occurs, it can be a serious problem due to differences in compounds and pharmacological effects. So it is very necessary to identify and discriminate plants that are closely related to ensure the quality, safety, and efficacy of raw materials. Related to the discrimination of plants of several varieties is still very limited, so a way is needed to distinguish several varieties of miana plants, especially if used as raw materials for traditional medicine. The required discrimination method should be fast, simple, and accurate.

The current method is used in discriminating a sample by using a combination method between spectroscopy and chemometrics. This combination of FT-IR spectral data with chemometric analysis with the aim of identification, authentication, and discrimination of closely related plants (Kucharska-Ambrożej et al., 2021; Umar et al., 2021; Truzzi et al., 2022; Umar et al., 2023). FT-IR spectroscopy can be an attractive option because it can meet the analysis criteria, to measure samples quickly without damage and analyse several components simultaneously (Truzzi et al., 2022; Umar et al., 2023a). In addition, analysis using FT-IR spectroscopy can provide information on the prediction of compounds by the absorption bands of functional groups that are specific to each compound, to distinguish a raw material that has similar compounds (Kucharska-Ambrożej et al., 2021; Rafi et al., 2021; Umar et al., 2021; Umar et al., 2023a).

The complex pattern of IR spectra makes direct and visual interpretation very complicated. To make it easier, chemometric techniques such as multivariate analysis are required (Umar et al., 2023a). Chemometric methods are used to find known statistical correlations of samples. Chemometric support expands the potential of FT-IR spectroscopy as an alternative method for analysing plant components (Umar et al., 2016). Commonly used chemometric methods to recognise and cluster sample patterns of both the same and different varieties are PCA, HCA, and PLS analyses (Rafi et al., 2021; Umar et al., 2021; Truzzi et al., 2022; Umar et al., 2023b). The grouping/discrimination of miana samples based on varieties using FT-IR and chemometric data has never been reported before, so this study aims to 1) analyse miana varieties using FT-IR, 2) identify sample groupings using the chemometric method, and 3) identify marker functional groups that can be used to distinguish miana samples.

MATERIAL AND METHODS

Materials

Samples of green (a), purple (b), batik (c), and combined colour (purple edge green) (d) miana leaves (Figure 1) were obtained from Salupao and Seriti villages, East Lamasi Sub-District, Luwu District, South Sulawesi. All samples were identified in the field based on published flora specimens and vouchers (B-235). Plant specimens are deposited at Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Cibinong, Indonesia.

Figure 1. Morphology of Miana plants: green (a), purple (b), batik (c) and combination (purple leaves with green edges) (d).

Methods

Sample Preparations

Sample preparation refers to Umar et al. (2023a) with slight modifications. Fresh miana leaves (the 3rd to 4th from the tip) that have been harvested, then dried using a simplicia oven at 40 ºC. The dried sample was then pulverized and sieved. Simplicia powder from each miana sample was weighed as much as 5 mg and mixed until homogeneous with 95 mg of KBr (5 repetitions) in a mortar. The mixture of KBr and simplicia powder was put into a pellet maker and then forged to form a disc (3 mm diameter). This process lasted for 10 minutes approximately and then the pellets were inserted into the sample container.

FT-IR spectroscopy analysis

FT-IR spectra of the samples were scanned using an FT-IR spectrophotometer (Nicolet™ iS10 FT-IR, Thermo Scientific™, USA), equipped with OMNIC™ software (Thermo Scientific™, USA). The analysis method refers to the method conducted by Umar et al., (2023a) in the range of wave numbers 4000–400 cm^{-1}. This analysis was used to obtain the spectrum pattern of the sample, then the sample wave number data and functional groups were analysed by chemometric techniques to identify, distinguish, classify, and detect each group of functional groups that might be potential markers for distinguishing miana varieties and functional groups from miana samples.

Data analysis

Wave number data processed from FT-IR analysis was processed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) for multivariate data analysis (MVDA) referring to Umar et al. (2023a). PLS-DA (wave number as variable X and functional group data as variable Y) was used to identify markers of functional groups among miana varieties. In addition, VIP scores were used to filter out potential marker functional groups in the sample. Sample normalisation using median and Pareto scaling (data scaling) was selected for PCA and PLS-DA analysis. The MVDA results were validated using permutation values (R^2 and Q^2) and VIP to confirm the reliability of the PLS-DA model.

RESULTS AND DISCUSSION

The FT-IR spectrum of miana samples has differences in the number of peaks, absorbance intensity, and wave numbers of each miana variety. The FT-IR spectrum profile of miana leaves (Figure 2) visually has the same pattern, but there are differences in intensity, especially at peaks 1, 2, 7, 8, 9, and 10. The absorbance intensity is very high in the purple miana sample (DMU) compared to all samples, especially the vibration of the –OH (phenol) group at 3200–3500 cm^{-1} (peak 1) (Rohman et al., 2021; Umar et al., 2023a). Peak 2 (3000–2850 cm^{-1}), showing vibrations of C–H groups (alkanes) (Puhan, Casford, & Davies, 2023). S=O (sulfone) group identified in the range of 1375–1300 cm^{-1} (peak 7) (Rogulska, 2023). Functional group of C–O (alcohol) in the range of 1300–1000 cm^{-1} (peak 8) (Liu et al., 2023a).

In addition, the difference is found in the 1000–650 cm^{-1} absorption band (peak 9 and 10), green miana and purple miana indicated the vibration of the =CH (aromatic) group (Maurya, Singh, & Rastogi, 2023) which is not found in miana batik and miana combined.

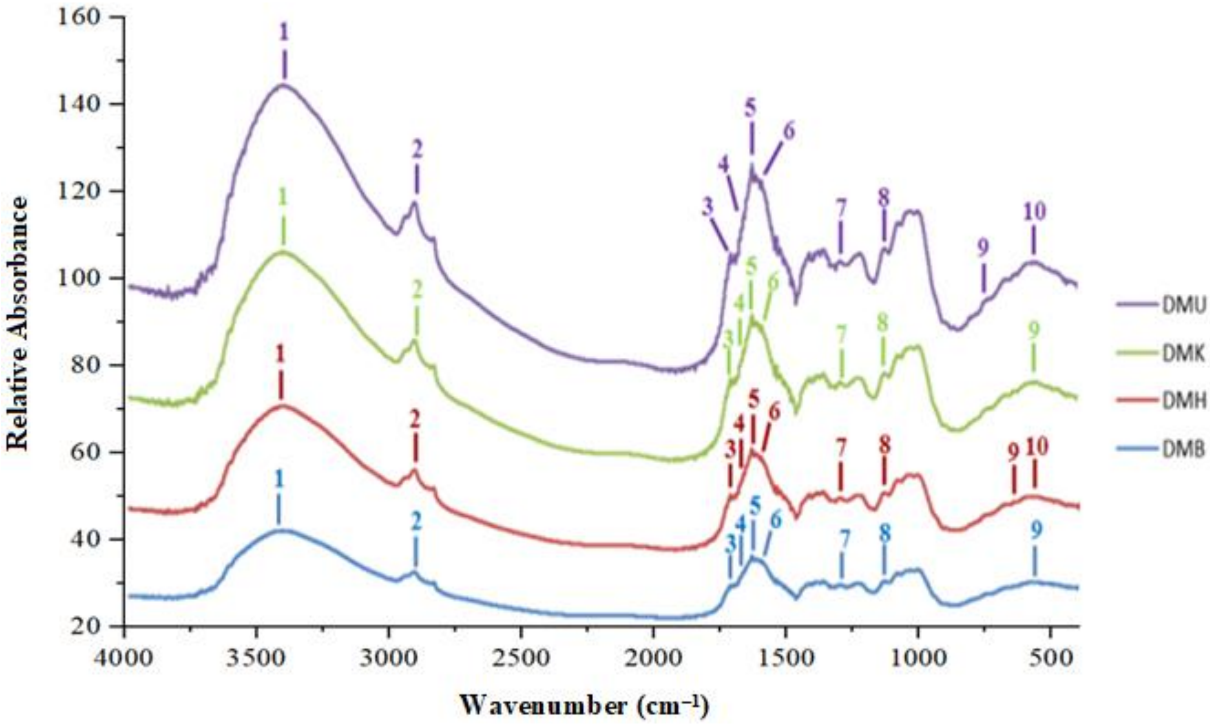

Figure 2. FT-IR spectra of purple miana (DMU), combined miana (DMK), green miana (DMH), and batik miana (DMB) samples in the wave number range 4000–400 cm^{-1}.

The results of PCA analysis are presented in Figure 3, obtained a PC-1 value of 97.7% and PC-2 of 1.8%. So in accumulation, from the results of PCA analysis using data on purple, combined, green, and batik miana leaf samples at wavelengths of 4000–400 cm^{-1} can explain the total variable of 99.5% of the sample data set. If the number of PC-1 and PC-2 is greater than 70%, then the results of the principal component plot show two dimensions of good sample discrimination (Tew et al., 2022; Zhan et al., 2022a; Zhang et al., 2022). PCA is a method often used in modelling the discrimination of closely related plants that belong to the unsupervised pattern recognition technique. The PC value provides information about the pattern contained in the sample. Plots for two initial PC values are usually most useful in analyses because these two PCs contain the most variation in the data (Kautsar et al., 2021; Rohman et al., 2021; Hegazi et al., 2022; Joshi et al., 2022; Losso et al., 2022; Zhan et al., 2022b). The PCA model has also been applied in differentiating three varieties of *Theobroma cacao* (Collazos-Escobar et al., 2023), *Triticum aestivum* (Liu et al., 2023b), *Brassica oleracea* (Langston et al., 2023).

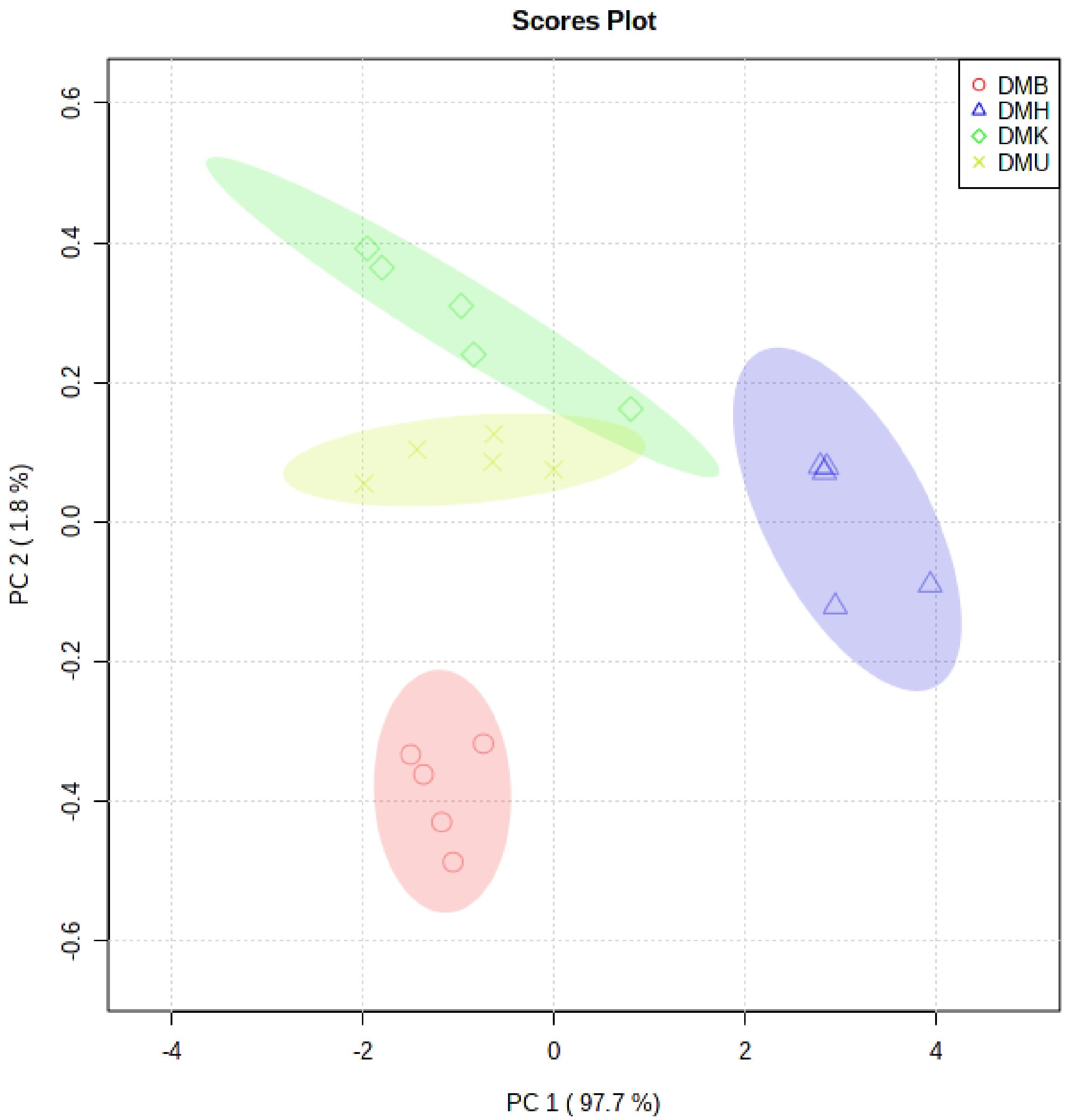

Figure 3. PCA score plots for principal components 1 (PC 1) and 2 (PC 2) of batik miana (DMB), green miana (DMH), combined miana (purple leaves with green edges) (DMK), and purple miana (DMU) using FT-IR wave number data.

Dendrogram results obtained from purple, combined, green, and batik miana leaf samples are divided into 4 groups (a, b, c, and d) at a distance of 2.5 (Figure 4). The first cluster (a) contained green miana (DMH), combined miana (DMK), and purple miana (DMU). Clusters two (b) and three (c) include combined miana (DMK) and purple miana (DMU). These results indicate that these samples have more similar physico-chemical profiles reflected in the infrared spectra. Meanwhile, the fourth cluster (d) contains batik miana (DMB). Clustering detection using the HCA model showed that discrimination between samples of the four miana leaf varieties was possible. Hierarchical cluster analysis (HCA) using dendrograms is carried out to observe similarities or differences between samples (Umar et al., 2023a).

This HCA method has been widely applied to identify samples based on sample differences and similarities, especially in the same genus and species (Umar et al., 2023b). Unsupervised Hierarchical Cluster Analysis (HCA) is a complement to PCA analysis. HCA is applied to determine the similarities and dissimilarities between each experimental sample. Samples with similar variables in the investigated variable are matched in the same cluster, but samples that show the highest dissimilarity are organized in another cluster (Idris et al., 2023). The HCA model has also been widely applied in discriminating samples such as *Artemisia argyi* varieties (Zhang et al., 2023), *Ocimum* species (Rai et al., 2023), and *Codonopsis* species (Liu et al., 2023c).

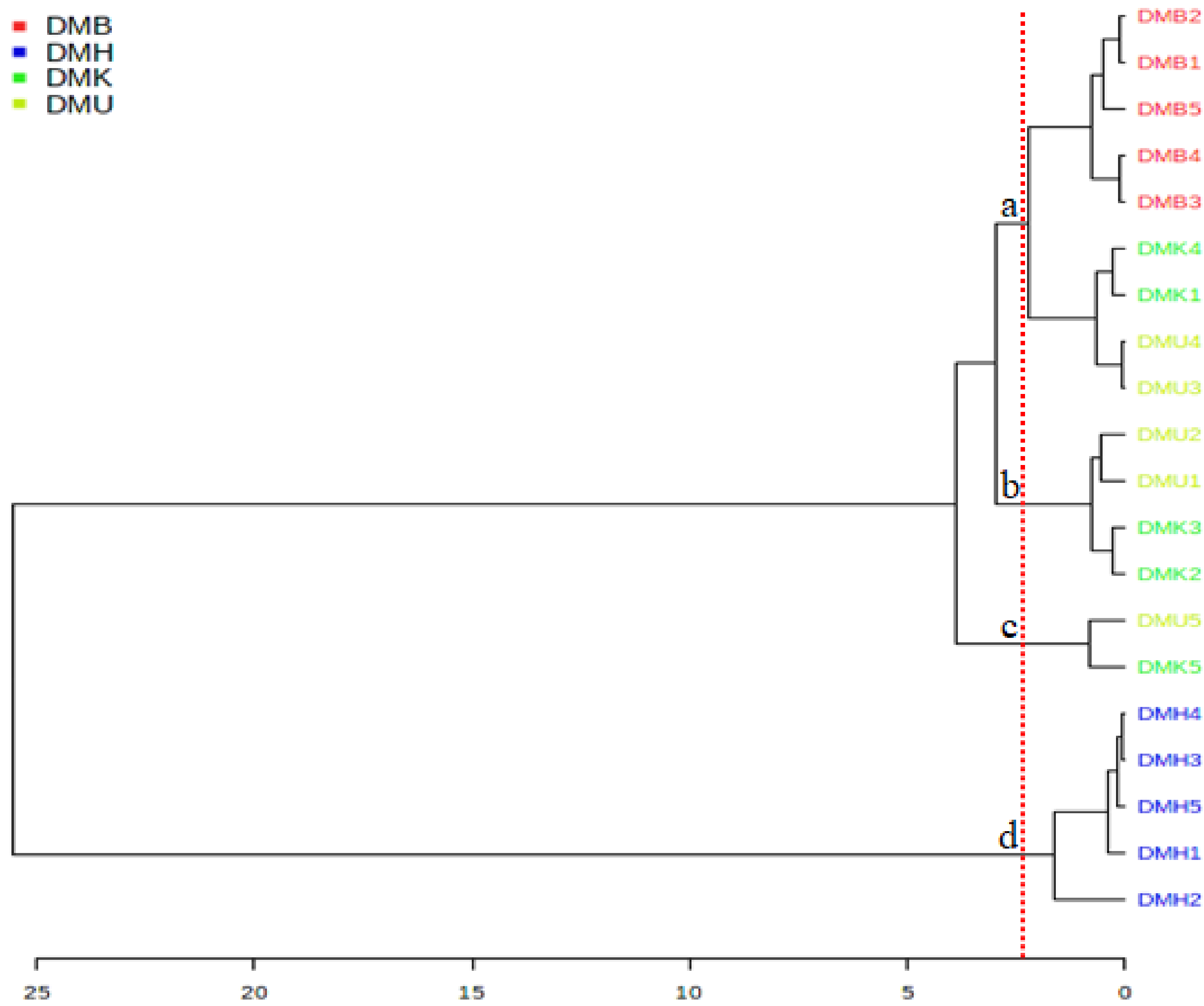

Figure 4. Dendrogram of sample varieties of batik miana (DMB), green miana (DMH), combination miana (DMK), and purple miana (DMU).

The results of the heatmap analysis of the four samples are presented in (Figure 5) using sigmoid curves (s-plot) and OPLS-DA. The colour intensity of the samples ranges from red (high) to blue (low), indicating the high and low intensity of the functional groups in each miana sample. Each row represents a functional group, and each column represents a sample. The heatmap results show that the green miana sample (DMH) has high peak absorbance intensity in the =CH, C=C, and C=O groups. C=C groups in DMU, DMB, DMH, and DMK. Heatmap analysis has been widely applied in identifying the abundance of functional groups and compounds in a sample (Mashiane et al., 2021; Umar et al., 2023a). Heatmaps illustrate the abundance of functional groups and compounds from each group of samples and can also be applied in grouping samples based on variety (Sachadyn-Król, Budziak-Wieczorek, & Jackowska, 2023; Umar et al., 2023a).

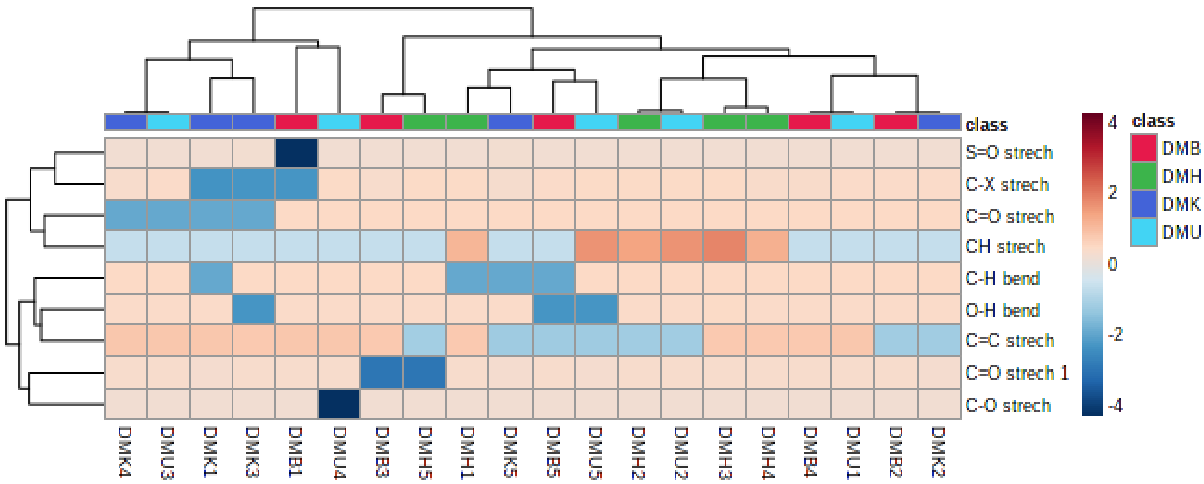

Figure 5. Heatmap between samples and functional groups of batik miana (DMB), green miana (DMH), combined miana (DMK), and purple miana (DMU) leaf samples. The colour bar (right) shows the intensity of the high (red) to low level (blue).

The results of the VIP score analysis are presented in Figure 6, showing the discriminant features identified using the PLS-DA model that were selected based on the scores of important variables that can be used as marker (discriminating) functional groups. The marker function clusters can be used to discriminate between the four miana varieties. A higher VIP value means it has a greater difference from other functional groups for varietal-level sample classification (Liu et al., 2023d). Features with a VIP score > 1 are considered important discriminators between samples, especially on functional groups and chemical compounds (Mashiane et al., 2021; Nguyen et al., 2023; Umar et al., 2023a). Discriminative functional groups of batik miana leaves, green miana leaves, combined miana leaves, and purple miana leaves with a VIP score > 1 include: C=O and C–H groups. The colour scale (right) indicates high to low intensity, red indicates higher absorbance intensity while blue indicates lower intensity. The VIP results show that the C=O stretching group was identified to have the highest intensity in the green miana leaf sample, while the C=O stretching group and C–H bending were identified to be the highest in the purple miana leaf sample compared to the other samples.

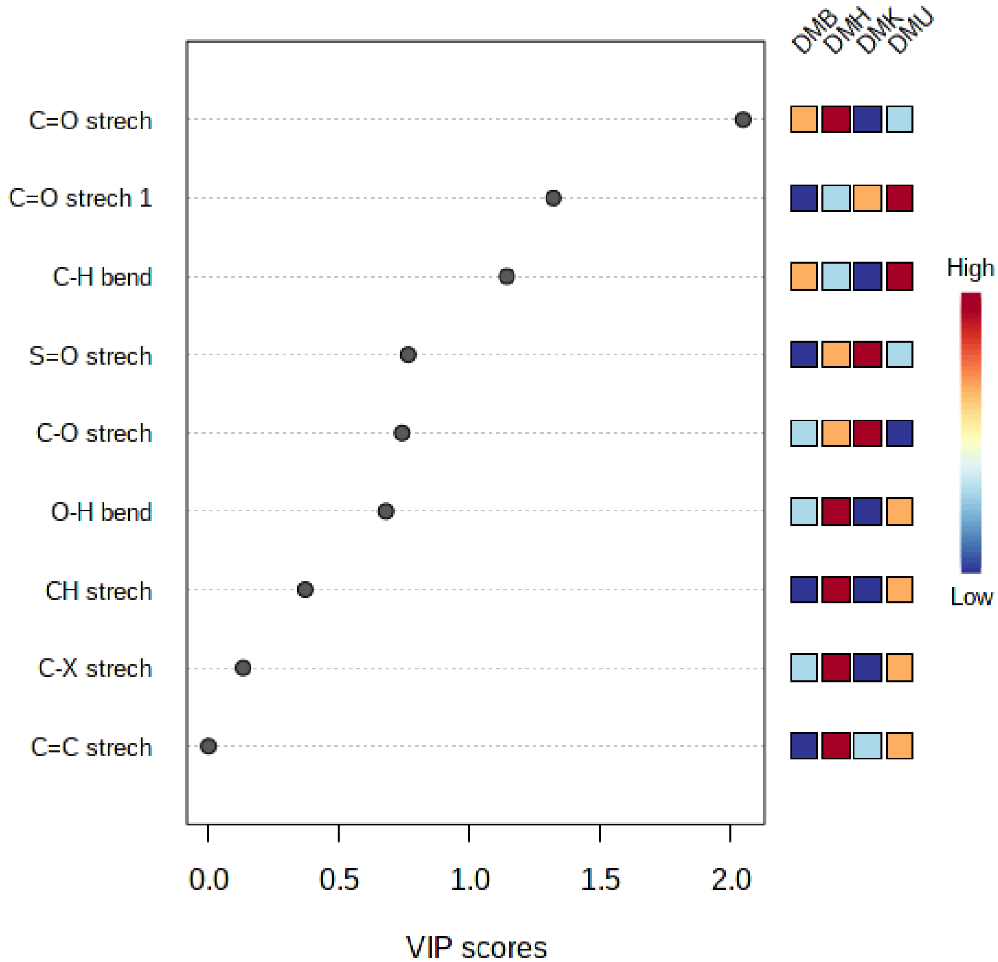

Figure 6. VIP scores of the functional groups of batik miana, green miana, combined miana, and purple miana leaf samples with intensity based on a colour scale from red (high) to blue (low).

CONCLUSION

Based on the research can be concluded that visually, the FT-IR spectra profiles of the miana species are identified as the same. The results of PCA, HCA, and OPLS-DA can discriminate the leaf samples of batik miana, green miana, combined miana, and purple miana. The total PCA value is 99.5% of the sample data set. HCA model, the miana samples are divided into four clusters, namely: a (DMB, DMK, and DMU), b (DMU and DMK), c (DMU and DMK), and d (DMH). The VIP score shows that the C=O group has the highest intensity in the green miana leaf sample, while the C=O stretch 1 and C–H bending groups are identified highest in the purple miana leaf sample compared to other samples. The combination of FT-IR spectroscopy and chemometric methods can be applied for miana variety discrimination. We conclude that the employment of pattern recognition algorithms is a useful tool, particularly for usage in the traditional medicine industry, for the characterisation and differentiation of miana species more specifically and medicinal plants in general. The use of FT-IR only identifies functional groups so that in the future a combination with LC-MS/MS and NMR instruments is needed to identify compounds that contribute to discriminating miana samples.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akaputra, R., Hatta, M., Massi, M. N., Djaharuddin, I., Bukhari, A., Aminuddin, A., … Azhar, A. (2023). Decreasing mRNA HMGB1 expression in *Klebsiella pneumoniae* infection treated by Miana (*Coleus scutellarioides* (L.) Benth): A cohort experimental study. *Annals of Medicine and Surgery*, *85*(6), 2395–2399. https://doi.org/10.1097/MS9.0000000000000908

Ayu, A. C., Ida, M., Moelyono, M., & Fakhriati, S. G. (2018). Total anthocyanin content and identification of anthocyanidin from *Plectranthus scutellarioides* (L.) R. Br leaves. *Research Journal of Chemistry and Environment*.

Aziz, P., Muhammad, N., Intisar, A., Abid, M. A., Din, M. I., Yaseen, M., … Ejaz, R. (2021). Constituents and antibacterial activity of leaf essential oil of *Plectranthus scutellarioides*. *Plant Biosystems*, *155*(6), 1247–1252. https://doi.org/10.1080/11263504.2020.1837279

Bismelah, N. A., Ahmad, R., Mohamed Kassim, Z. H., Ismail, N. H., & Rasol, N. E. (2022). The antibacterial effect of *Plectranthus scutellarioides* (L.) R.Br. Leaves extract against bacteria associated with peri-implantitis. *Journal of Traditional and Complementary Medicine*, *12*(6), 556–566. https://doi.org/10.1016/j.jtcme.2022.07.002

Collazos-Escobar, G. A., Barrios-Rodriguez, Y. F., Bahamón-Monje, A. F., & Gutiérrez-Guzmán, N. (2023). Uses of mid-infrared spectroscopy and chemometric models for differentiating between dried cocoa bean varieties. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *27*, 803–810. https://doi.org/10.1590/1807-1929/agriambi.v27n10p803-810

Dörr, O. S., Zimmermann, B. F., Kögler, S., & Mibus, H. (2019). Influence of leaf temperature and blue light on the accumulation of rosmarinic acid and other phenolic compounds in *Plectranthus scutellarioides* (L.). *Environmental and Experimental Botany*, *167*, 103830. https://doi.org/10.1016/j.envexpbot.2019.103830

Hegazi, N. M., Khattab, A. R., Frolov, A., Wessjohann, L. A., & Farag, M. A. (2022). Authentication of saffron spice accessions from its common substitutes via a multiplex approach of UV/VIS fingerprints and UPLC/MS using molecular networking and chemometrics. *Food Chemistry*, *367*, 130739. https://doi.org/10.1016/j.foodchem.2021.130739

Idris, N. S., Khandaker, M. M., Rashid, Z. M., Majrashi, A., Alenazi, M. M., Adnan, A. F. M., … Mat, N. (2023). Discrimination of *Syzygium samarangense* cv. ‘Giant Green’ leaves at different maturity stages by FTIR and GCMS fingerprinting. *Horticulturae*, *9*(5), 609. https://doi.org/10.3390/horticulturae9050609

Joshi, R., Sathasivam, R., Park, S. U., Lee, H., Kim, M. S., Baek, I., & Cho, B.-K. (2022). Application of fourier transform infrared spectroscopy and multivariate analysis methods for the nondestructive evaluation of phenolics compounds in Moringa powder. *Agriculture*, *12*(1), 10. https://doi.org/10.3390/agriculture12010010

Kautsar, A., Wahyuni, W. T., Syafitri, U. D., Muflihah, S., Mawadah, N., Rohaeti, E., … Rafi, M. (2021). Data fusion of UV-Vis and FTIR spectra combined with principal component analysis for distinguishing of Andrographis paniculata extracts based on cultivation ages and solvent extraction. *Indonesian Journal of Chemistry*, *21*(3), 753–760. https://doi.org/10.22146/ijc.60321

Kucharska-Ambrożej, K., Martyna, A., Karpińska, J., Kiełtyka-Dadasiewicz, A., & Kubat-Sikorska, A. (2021). Quality control of mint species based on UV-VIS and FTIR spectral data supported by chemometric tools. *Food Control*, *129*, 108228. https://doi.org/10.1016/j.foodcont.2021.108228

Langston, F., Redha, A. A., Nash, G. R., Bows, J. R., Torquati, L., Gidley, M. J., & Cozzolino, D. (2023). Qualitative analysis of broccoli (*Brassica oleracea* var. Italica) glucosinolates: Investigating the use of mid-infrared spectroscopy combined with chemometrics. *Journal of Food Composition and Analysis*, *123*, 105532. https://doi.org/10.1016/j.jfca.2023.105532

Liu, M., Lei, Z., Cao, X., Yan, J., Shui, H., Wang, Z., … Hong, M. (2023a). Construction of macromolecules of depolymerized lignite. *ACS Omega*, *8*(25), 22820–22826. https://doi.org/10.1021/acsomega.3c01768

Liu, H.-Y., Wadood, S. A., Xia, Y., Liu, Y., Guo, H., Guo, B.-L., & Gan, R.-Y. (2023b). Wheat authentication: An overview on different techniques and chemometric methods. *Critical Reviews in Food Science and Nutrition*, *63*(1), 33–56. https://doi.org/10.1080/10408398.2021.1942783

Liu, X., Chen, Z., Wang, X., Luo, W., & Yang, F. (2023c). Quality assessment and classification of *Codonopsis* radix based on fingerprints and chemometrics. *Molecules*, *28*(13), 5127. https://doi.org/10.3390/molecules28135127

Liu, H., Yu, Y., Zou, B., Yu, Y., Yang, J., Xu, Y., … Yang, F. (2023d). Evaluation of dynamic changes and regularity of volatile flavor compounds for different green plum (*Prunus mume* Sieb. Et Zucc) varieties during the ripening process by HS-GC–IMS with PLS-DA. *Foods*, *12*(3), 551. https://doi.org/10.3390/foods12030551

Losso, K., Bec, K. B., Mayr, S., Grabska, J., Stuppner, S., Jones, M., … Huck, C. W. (2022). Rapid discrimination of Curcuma longa and Curcuma xanthorrhiza using direct analysis in real time mass spectrometry and near infrared spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *265*, 120347. https://doi.org/10.1016/j.saa.2021.120347

Mashiane, P., Manhivi, V. E., Shoko, T., Slabbert, R. M., Sultanbawa, Y., & Sivakumar, D. (2021). Cooking african pumpkin leaves (*Momordica balsamina* L.) by stir-frying improved bioactivity and bioaccessibility of metabolites-metabolomic and chemometric approaches. *Foods*, *10*(11), 2890. https://doi.org/10.3390/foods10112890

Maurya, A., Singh, R., & Rastogi, S. (2023). Study of vibrational spectra of polycyclic aromatic hydrocarbons with phenyl side group. *Journal of Molecular Spectroscopy*, *391*, 111720. https://doi.org/10.1016/j.jms.2022.111720

Nguyen, H. T., Phan, L. K., Huynh, K.-L. V., Duong, T.-H., Le, H. T., Hai-Yen, N. T., … Nguyen, M. D. (2023). Untargeted metabolomics approach for the differentiation between *Panax vietnamensis* var. Vietnamensis and *Panax vietnamensis* var. Fuscidiscus. *Metabolites*, *13*(6), 763. https://doi.org/10.3390/metabo13060763

Puhan, D., Casford, M. T. L., & Davies, P. B. (2023). Evaluation of structural and compositional changes of a model monoaromatic hydrocarbon in a benchtop hydrocracker using GC, FTIR, and NMR spectroscopy. *ACS Omega*, *8*(39), 35988–36000. https://doi.org/10.1021/acsomega.3c03833

Rafi, M., Rismayani, W., Sugiarti, R. M., Syafitri, U. D., Wahyuni, W. T., & Rohaeti, E. (2021). FTIR-based fingerprinting combined with chemometrics for discrimination of *Sonchus arvensis* leaves extracts of various extracting solvents and the correlation with its antioxidant activity. *Indonesian Journal of Pharmacy*, 132–140. https://doi.org/10.22146/ijp.755

Rai, A. K., Khan, S., Kumar, A., Dubey, B. K., Lal, R. K., Tiwari, A., … Ch, R. (2023). Comprehensive Metabolomic Fingerprinting Combined with Chemometrics Identifies Species- and Variety-Specific Variation of Medicinal Herbs: An Ocimum Study. *Metabolites*, *13*(1), 122. https://doi.org/10.3390/metabo13010122

Rogulska, M. (2023). The influence of diisocyanate structure on thermal stability of thermoplastic polyurethane elastomers based on diphenylmethane-derivative chain extender with sulfur atoms. *Materials*, *16*(7), 2618. https://doi.org/10.3390/ma16072618

Rohman, A., Ikhtiarini, A. N., Setyaningsih, W., Rafi, M., Aminah, N. S., Insanu, M., … Santosa, D. (2021). The use of chemometrics for classification of sidaguri (*Sida rhombifolia*) based on FTIR spectra and antiradical activities. *Indonesian Journal of Chemistry*, *21*(6), 1568–1576. https://doi.org/10.22146/ijc.64360

Sachadyn-Król, M., Budziak-Wieczorek, I., & Jackowska, I. (2023). The Visibility of Changes in the Antioxidant Compound Profiles of Strawberry and Raspberry Fruits Subjected to Different Storage Conditions Using ATR-FTIR and Chemometrics. *Antioxidants*, *12*(9), 1719. https://doi.org/10.3390/antiox12091719

Silalahi, M., Nisyawati, Walujo, E. B., Supriatna, J., & Mangunwardoyo, W. (2015). The local knowledge of medicinal plants trader and diversity of medicinal plants in the Kabanjahe traditional market, North Sumatra, Indonesia. *Journal of Ethnopharmacology*, *175*, 432–443. https://doi.org/10.1016/j.jep.2015.09.009

Tambaru, E., Ura', R., & Tuwo, M. (2023). Diversity of herbal medicine in Mamasa District, West Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity*, *24*(4). https://doi.org/10.13057/biodiv/d240410

Tew, W. Y., Ying, C., Wujun, Z., Baocai, L., Yoon, T. L., Yam, M. F., & Jingying, C. (2022). Application of FT-IR spectroscopy and chemometric technique for the identification of three different parts of *Camellia nitidissima* and discrimination of its authenticated product. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.931203

Truzzi, E., Durante, C., Bertelli, D., Catellani, B., Pellacani, S., & Benvenuti, S. (2022). Rapid classification and recognition method of the species and chemotypes of essential oils by ATR-FTIR spectroscopy coupled with chemometrics. *Molecules*, *27*(17), 5618. https://doi.org/10.3390/molecules27175618

Umar, Abd Halim, Syahruni, R., Burhan, A., Maryam, F., Amin, A., Marwati, M., & Masero, L. R. (2016). Determinasi dan analisis finger print tanaman murbei (*Morus alba* Lour) sebagai bahan baku obat tradisional dengan metode spektroskopi FT-IR dan kemometrik. *Pharmacon*, *5*(1). https://doi.org/10.35799/pha.5.2016.11227

Umar, Abdul Halim, Ratnadewi, D., Rafi, M., & Sulistyaningsih, Y. C. (2021). Untargeted metabolomics analysis using FTIR and UHPLC-Q-Orbitrap HRMS of two *Curculigo* species and evaluation of their antioxidant and α-glucosidase inhibitory activities. *Metabolites*, *11*(1), 42. https://doi.org/10.3390/metabo11010042

Umar, Abdul Halim, Ratnadewi, D., Rafi, M., Sulistyaningsih, Y. C., & Hamim, H. (2023). Phenolics profile and antioxidant activities of in vitro propagules and field-raised plant organs of *Curculigo latifolia*. *Journal of Applied Pharmaceutical Science*, *13*,(4), 168–185. https://doi.org/10.7324/JAPS.2023.55995

Umar, Abdul Halim, Syahruni, R., Ranteta'dung, I., & Rafi, M. (2023). FTIR-based fingerprinting combined with chemometrics method for rapid discrimination of *Jatropha* spp. (Euphorbiaceae) from different regions in South Sulawesi. *Journal of Applied Pharmaceutical Science*, *13*,(1), 139–149. https://doi.org/10.7324/JAPS.2023.130113

Zhan, W., Yang, X., Lu, G., Deng, Y., & Yang, L. (2022a). A rapid quality grade discrimination method for *Gastrodia elata* powder using ATR-FTIR and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *264*, 120189. https://doi.org/10.1016/j.saa.2021.120189

Zhan, W., Yang, X., Lu, G., Deng, Y., & Yang, L. (2022b). A rapid quality grade discrimination method for Gastrodia elata powderusing ATR-FTIR and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *264*, 120189. https://doi.org/10.1016/j.saa.2021.120189

Zhang, L., Wei, Y., Wang, W., Fan, Y., Li, F., Li, Z., … Li, X. (2023). Quantitative fingerprint and antioxidative properties of *Artemisia argyi* leaves combined with chemometrics. *Journal of Separation Science*, *46*(5), e2200624. https://doi.org/10.1002/jssc.202200624

Zhang, Y.-C., Deng, J., Lin, X.-L., Li, Y.-M., Sheng, H.-X., Xia, B.-H., & Lin, L.-M. (2022). Use of ATR-FTIR spectroscopy and chemometrics for the variation of active components in different harvesting periods of *Lonicera japonica*. *International Journal of Analytical Chemistry*, *2022*, e8850914. https://doi.org/10.1155/2022/8850914