# Fingerprint Metabolite of Miana (*Coleus* sp.) Leaf Infusion and Juice: Authentication Based on FTIR Spectroscopy and Multivariate Analysis

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#### ABSTRACT

Miana (*Coleus* sp.) is a plant belonging to the Lamiaceae family and has been widely used in traditional medicine. Despite the potential of the plant, the quality and quantity of chemical components that can be extracted are dependent on various factors, such as varieties and extraction techniques. Therefore, this study aimed to discriminate miana based on varieties and extraction techniques as well as identify its marker functional groups. The 4 different leaf varieties of the plant, including purple, green, batik, and color combination were extracted using the infusion method, both with and without the addition of water. FTIR (Fourier transform infrared) fingerprint metabolite of each extract was then analyzed with IRPal 2.0 software for functional group interpretation and multivariate data analysis (PCA and PLS-DA) using MetaboAnalyst 5.0. The results showed that the chromatogram profile of all varieties and extraction techniques had similar patterns. The PCA (principal component analysis) score plot yielded a total PC value of 98.5%, which could effectively discriminate the samples. In addition, the O–H group's VIP score value (greater than 1) was a significant functional group that characterized the samples. Based on these results, the combination of FTIR spectroscopy and multivariate analysis could be used to discriminate miana based on varieties and extraction techniques, as well as identify functional groups serving as marker.

Keywords: Chemometrics; Coleus sp.; extraction; FTIR; fingerprint metabolite

#### INTRODUCTION

Miana (*Coleus scutellarioides* (L.) Benth.). belonging to the Lamiaceae is a plant growing in almost all regions of Indonesia. The plant has different leaf pigments, colors (green to pink), and shapes (triangular or ovate). In addition, Indonesians have widely used miana for various purposes, including as a medicinal ingredient (Auliawan & Cahyono, 2015; Marpaung et al., 2014), as a ritual complement (Hidayat et al., 2016; Suswita, 2013), and as an ornamental plant (Haryanti et al., 2015; Hidayat et al., 2016).

According to previous studies, people often use miana traditionally by grinding until smooth and then

mixing with drinking water. The leaf or stem of the plant can also be boiled and then drunk (AgroMedia, 2008), to treat asthma, boils, diarrhea, worms, and coughs with phlegm, as well as smoothing the menstrual cycle, neutralizing toxins, and increasing appetite (Kusumawati et al., 2014). Organoleptically, its leaf has a distinctive aroma (fragrant), tastes slightly bitter, and is cold, because of its chemical content, such as minerals, ethyl salicylate, methyl eugenol, carvacrol thymol, calcium oxalate, fat, phytosterols, and alkaloids (Pusat Studi Biofarmaka LPPM IPB & Gagas Ulung, 2014).

Plants typically contain varying compounds, hence, analytical techniques, such as FTIR spectroscopy (Fourier transform infrared) and chemometrics are

DOI: http://doi.org/10.22146/agritech.88706 ISSN 0216-0455 (Print), ISSN 2527-3825 (Online) necessary (Umar et al., 2016; Umar, et al., 2023a; Lestari et al., 2024). These techniques generally combine FTIR spectrum data with chemometrics for identification, discrimination, and sample authentication (Purwakusumah et al., 2014; Puspitasari et al., 2021; Noviany et al., 2023), as well as predicting pharmacological activity from various organs (Umar et al., 2021). In addition, relative plants are very difficult to identify using only morphological observations due to similarities. Using FTIR spectroscopy and chemometrics together helps to tell samples apart based on their group, type, breed, and species (Aouidi et al., 2012; Cai et al., 2015; Di Donato et al., 2020; Kucharska-Ambrożej et al., 2021). These methods are also used to find fake traditional medicines when the main ingredients are the same (Kucharska-Ambrożej & Karpinska, 2020; Umar et al., 2016).

Several studies suggest that FTIR spectroscopy is a dependable method for analyzing the chemical traits of materials. This is mostly because it is a fast, simple, and safe way to test samples without damage (Umar et al., 2021). Even if the chemical makeup of the samples is not known, the spectrum obtained can still help in the separation process (Sun et al., 2010). To interpret the infrared patterns, chemometrics are usually used to provide clearer information (Rohman et al., 2014; Umar et al., 2021). Therefore, this study aimed to differentiate miana based on varieties and extraction methods as well as identify its marker functional groups. The results are expected to serve as a foundation for classifying important functional group-based fingerprint and chemometric parameters in miana varieties with high accuracy and precision, specifically in the authentication of traditional

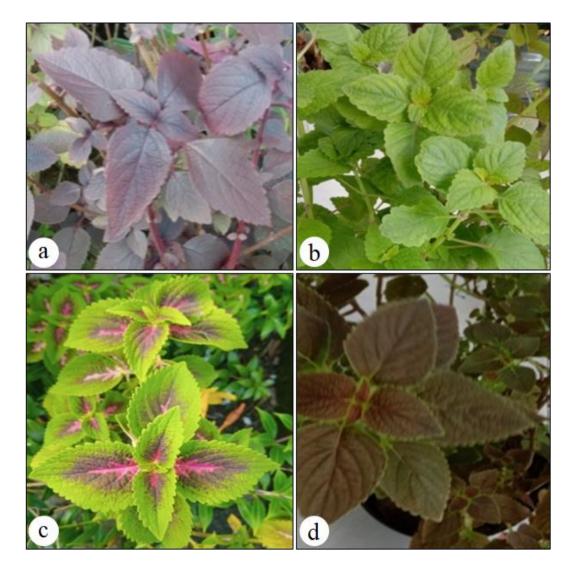


Figure 1. Morphology of miana (*Coleus scutellarioides* (L.) Benth.) Purple miana (a), green miana (b), batik miana (c), and color combination miana (d) varieties.

medicine raw materials and other fields of natural product chemistry.

# METHODS

## Materials

Cultivated miana *(Coleus scutellarioides)* samples namely green miana (a), purple miana (b), batik miana (c), and color combination miana (d) (Figure 1) with ages ranging from 4–5 months, were collected from Balaikembang Village, Mangkutana Subdistrict, East Luwu Regency, and South Sulawesi (2°21'18"S, 120°38'22"E). The plant part used was a fresh leaf (3-4<sup>th</sup> leaf from the shoot), and all samples were identified in the field based on flora specimens and issued vouchers. Plant specimens were stored at the Herbarium Bogoriense, National Report and Innovation Agency (BRIN), Cibinong, Indonesia. Voucher samples were also stored in the Herbarium of Almarisah Madani University, Makassar, South Sulawesi.

## **Sample Preparation**

This study used fresh samples of miana (*Coleus scutellarioides*) (green miana, purple miana, batik miana, and color combination miana), and the infusion method referred to by Francik et al., (2020) with several modifications. To make an infusion, 10 grams of sliced miana leaves were heated with 100 milliliters of distilled water until the temperature reached 90 degrees Celsius and maintained for 15 minutes. After heating, filter paper was used to separate the solid ingredients from the liquid. Then, another similar batch of leaves and water was added to the container for the same process. This process produced 4 separate infusions, bastik miana leaf (BIF), green miana leaf infusion (HIF), colorful miana leaf infusion (KIF), and purple miana leaf infusion (UIF).

Two methods were used to extract juice from miana leaves. In the first method, 10 grams of fresh leaves were crushed in a mortar until smooth, then 100 milliliters of distilled water was added, and the mixture was ground until it was uniform. The material was then crushed further and filtered through filter paper before being mixed again with 100 milliliters of distilled water. In the second method, 10 grams of miana leaves were crushed using a mortar and pestle until they reached a smooth consistency. After crushing, the leaf paste was pressed and then filtered through filter paper. At this time, miana leaf juice was mixed with water to produce batik (BPA), purple (UPA), green (HPA), and color combination (KPA) miana leaf juice. Additionally, several juices were available without the need for water addition, including batik (BPE), green (HPE), color combination (KPE), and purple (UPE) miana leaf juice.

External validation of each sample was made by mixing samples from each method (infusion, juice with water, and juice without water in the same ratio), including batik (BVA), green (HVA), color combination (KVA), and purple (UVA) miana leaf validation. The infusion and juice filtrates (with water and without water) with a liquid consistency from each miana variety and their validation were further analyzed using FTIR.

# FTIR Spectroscopic Analysis

The FTIR spectrum of the samples was scanned using an FTIR spectrophotometer (Nicolet<sup>™</sup> iS10 FTIR, Thermo Scientific<sup>™</sup>, USA), equipped with OMNIC<sup>™</sup> software (Thermo Scientific™, USA). Measurements were carried out using horizontal attenuated total reflectance (HATR) equipped with a germanium crystal in the wave number ranged  $4,000-400 \text{ cm}^{-1}$  (16 scans, resolution 16 cm<sup>-1</sup>, and interval 1,928 cm<sup>-1</sup>). All FTIR spectra were corrected against the FTIR spectrum of air as background, and measurements were carried out in 5 repetitions as absorbance values. FTIR analysis was used as a qualitative analysis to analyze the functional groups of miana samples using IRPal 2.0 (https:// irpal.software.informer.com/2.0/) and OriginLab. This analysis was used to obtain a spectrum pattern from the sample and the functional groups were analyzed using chemometric techniques to identify, differentiate, group, and detect each group of functional groups which could be potential markers for distinguishing varieties and functional groups from miana samples (Umar et al., 2023a).

## **Data Analysis**

Wavenumber data (FTIR data) were analyzed with chemometric techniques using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). PCA (principal component analysis) was performed for sample similarities, and differences between grouping, varieties and extraction methods. Furthermore, PLS-DA (spectra data as variable X and functional group data as variable Y) was used to identify marker functional groups between miana varieties. Furthermore, the VIP score was used to screen potential marker functional groups in the sample., and the normalization using median and Pareto scaling (data scaling) was selected for PCA and PLS-DA analysis. MVDA results were validated using permutation values (R<sup>2</sup> and Q<sup>2</sup>) and VIP to confirm the reliability of the PLS-DA model (Umar et al., 2023a).

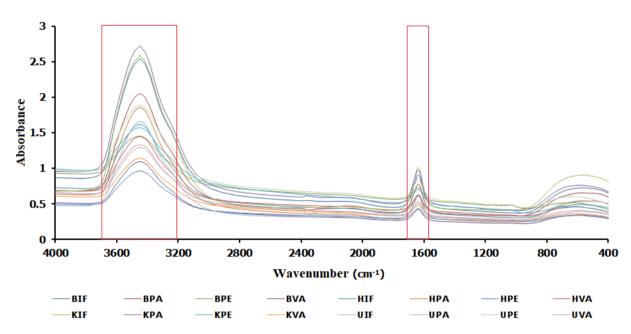


Figure 2. FTIR spectrum of Miana leaf extract from 4 varieties using 3 extraction methods. BIF, batik miana leaf infusion, BPA, batik miana leaf juice with water, BPE, batik miana leaf juice without water, BVA, batik miana leaf validation, HIF, green miana leaf infusion, HPA, green miana leaf juice with water, HVE, green miana leaf juice without water, HVA, green miana leaf validation, KIF, color combination miana leaf infusion, KPE, color combination miana leaf validation, UIF, purple miana leaf infusion, UPA, purple miana leaf juice with water, UPE, purple miana leaf juice without water, and UVA, purple miana leaf validation. Sample validation consisted of a mixture of infusion, juice with water, and without water.

## **RESULTS AND DISCUSSION**

The chromatogram profile of miana leaf based on the variety and extraction method used had a distinctive spectrum pattern (sample absorbance intensity), visually showing the similarities of each sample. A comparison of the spectrum profiles of each miana leaf extract sample was presented in Figure 2. The FTIR spectrum of miana samples had differences in absorbance intensity, namely at wavelengths of 3400 cm<sup>-1</sup> (O–H) and 1650 cm<sup>-1</sup> (C–C). This difference in intensity indicated a distinction in compound levels in the sample (Umar et al., 2023a).

PCA or unsupervised pattern recognition was a method commonly used to discriminate samples within a (closely related) species (Noviany et al., 2023; Umar et al., 2023a). Chemometric techniques, in the form of PCA, had also been reported to be able to discriminate samples of tea varieties (Cai et al., 2015), *Curcuma* (Wang et al., 2021), and rice (Rahmani & Mani-Varnosfaderani, 2022). The PC value provided information regarding similarities and differences in samples (Rafi et al., 2023). The value information used came from PC-1 and PC-2 (capable of interpreting as many data variables as possible) (Puspitasari et al., 2021). The results of the PCA analysis (Figure 3) showed that the total PC value was 98.5% (PC-1 was 79.6% and PC-2 was 18.9%). According to Umar et al. (2023a), when the total PC value was above 70%, this indicated a good prediction model.

The dendrogram analysis in this study was aimed at examining the grouping of miana batik, green, color combination, and purple samples with 3 extraction treatments. The dendrogram (Figure 4) formed indicated 3 sample clusters (at a distance of 100). This showed that the sample extraction process influenced sample grouping, by producing clusters based on similarity patterns. A large similarity (the same cluster) indicated a higher similarity, and vice versa (Umar et al., 2023b). The differences showed that the same variety using different extraction processes had differences. Similarities and differences in samples based on varieties using dendrograms had also been applied to apricot varieties (Basile et al., 2022) and rice (Giang et al., 2020).

The results of the heatmap analysis were presented in Figure 5. In the heatmap, the sample absorbance intensity of several functional groups

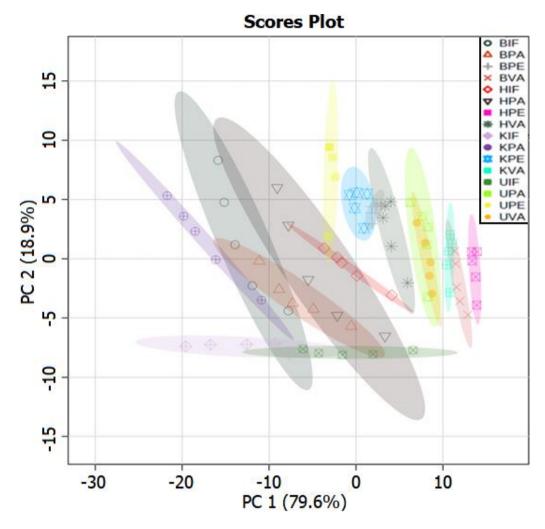


Figure 3. PCA plot score values (PC-1 and PC-2) of miana leaf extract samples. BIF, Batik miana leaf infusion, BPA, Batik miana leaf juice with water, BPE, Batik miana leaf juice without water, BVA, Batik miana leaf validation. HIF, green miana leaf infusion, HPA, green miana leaf juice with water, HPE, green miana leaf juice without water, HVA, green miana leaf validation. KIF, color combination miana leaf infusion, KPE, color combination miana leaf juice without water, KPA, color combination miana leaf juice with water, KVA, color combination miana leaf infusion, UPA, purple miana leaf juice with water, UPE, purple miana leaf juice without water, and UVA, purple miana leaf validation.

was represented by brown (high) to blue (low) color intensity. The highest intensity of each functional group was identified in samples of KIF (color combination miana leaf infusion), KPA (color combination miana leaf juice with water), and BIF (miana leaf batik infusion). Heatmap analysis could differentiate samples (especially the intensity of compounds and functional groups) based on different extraction methods and filter fluids used, as well as differences in species (Rafi et al., 2023; Umar et al., 2023a), this was also observed in this study, especially in its functional group. The results of the PLS-DA analysis were VIP scores (Figure 6), and samples were shown with a color code scaled from red (high) to blue (low). The higher the VIP score (> 1), the higher the impact of functional groups/metabolites as a differentiator (Umar et al., 2023a). Functional groups (O–H Phenol) with a VIP value > 1 were considered important in distinguishing purple, green, batik, and color combination miana. Several species of *Jatropha* (Umar et al., 2023a) and lettuce (Falcioni et al., 2022) had also been reported to have functional groups as inter-species identifiers using the PLS-DA method.

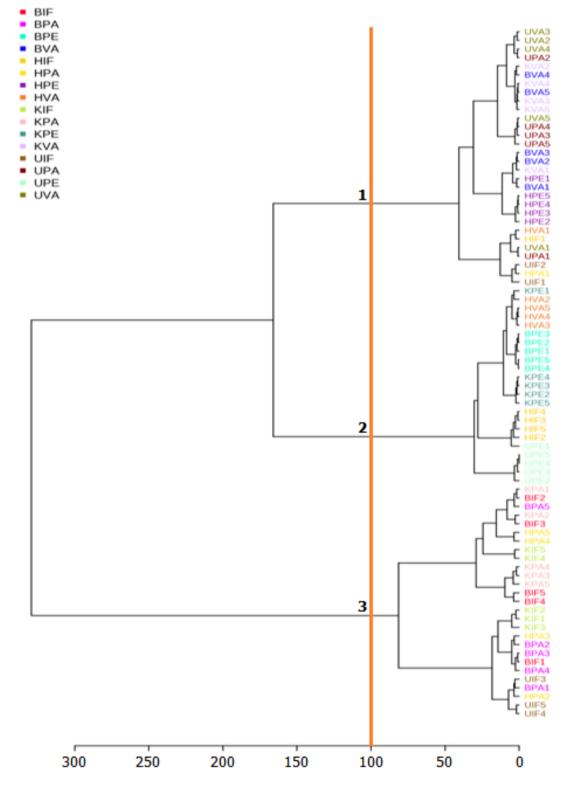


Figure 4. Dendrogram of the similarity relationship between miana leaf extracts from 4 leaf types and 3 extraction treatments. BIF, Batik miana leaf infusion, BPA, Batik miana leaf juice with water, BPE, Batik miana leaf juice without water. HIF, green miana leaf infusion, HPA, green miana leaf juice with water, HPE, green miana leaf juice without water. KIF, color combination miana leaf infusion, KPA, color combination miana leaf juice with water, KPE, color combination miana leaf juice without water, UIF, purple miana leaf infusion, UPA, purple miana leaf juice with water, UPE, purple miana leaf juice without water, and UVA, purple miana leaf validation.

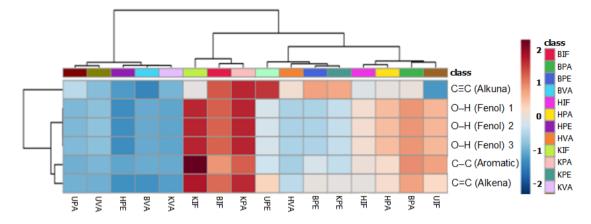


Figure 5. Heatmap, colored areas on the heatmap correspond to different sample concentrations. BIF, Batik miana leaf infusion, BPA, Batik miana leaf juice with water, BPE, Batik miana leaf juice without water, BVA, Batik miana leaf validation. HIF, green miana leaf infusion, HPA, green miana leaf juice with water, HPE, green miana leaf juice without water, HVA, green miana leaf validation. KIF, color combination miana leaf infusion, KPE, color combination miana leaf juice with water, KVA, color combination miana leaf juice with water, KVA, color combination of miana leaf validation, UIF, purple miana leaf infusion, UPA, purple miana leaf juice with water, UPE, purple miana leaf juice without water, and UVA, purple miana leaf validation.

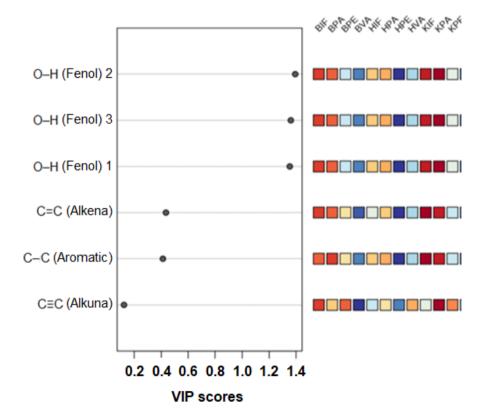


Figure 6. VIP scores of functional groups of miana samples. BIF, Batik miana leaf infusion, BPA, Batik miana leaf juice with water, BVA, Batik miana leaf validation. HIF, green miana leaf infusion, HPA, green miana leaf juice with water, HPE, green miana leaf juice without water, HVA, green miana leaf validation. KIF, color combination miana leaf infusion, KPE, color combination miana leaf juice with water, KPA, color combination miana leaf juice with water, KVA, color combination of miana leaf validation, UIF, purple miana leaf infusion, UPA, purple miana leaf juice with water, UPE, purple miana leaf juice without water, and UVA, purple miana leaf validation.

## CONCLUSION

In conclusion, the application of the FTIR spectroscopy method combined with multivariate analysis could discriminate/group miana samples based on varieties and simple extraction methods (infusion, juice with the addition of water, and juice without water) with a total PC value of 98.5% (PC-1: 79.6% and PC-2: 18.9%). The marker functional group that differentiated each sample was the O-H group. In addition, the combination of FTIR spectroscopy and chemometrics could be used in the traditional medicine industry, for more specific characterization and differentiation of varieties and medicinal plants in general. The use of FTIR only identified functional groups, therefore in the future, a combination with NMR and LC-HRMS instruments was needed to identify compounds that contributed to differentiating miana varieties as well as various other chemometrics techniques.

## **CONFLICT OF INTEREST**

There was no conflict of interest in this study.

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