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# Direct FTIR and Chemometrics for Authentication of Kratom Powder and Other Alkaloids-containing Plant Matters

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ABSTRACT

Kratom (*Mitragyna speciosa*), has gained attention for its use as stimulant and opioid-like analgesic effects. In Indonesia, its legal status remains uncertain in many regions, and concerns about its safety have led to increasing regulation. Even with uncertainty with its legal status, kratom remains easily accessed via online market. However with Indonesia's Ministry of Trade is set to regulate its export regulation, a method to quickly distinguish kratom venations is needed. This study explores the potential of Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy, combined with chemometric techniques like Principal Component Analysis (PCA), to differentiate between kratom venations and other alkaloid-containing plants. Direct ATR-FTIR analysis of ground kratom, tea, and coffee leaves revealed characteristic functional groups, with distinct spectral variations observed across the plant samples. Cluster variable analysis reduced the dimensionality of the spectral data by 98.6%, while maintaining 98% similarity level. PCA highlighting key principal components (PC1 and PC2) responsible for 93.9% of the variance. The model successfully grouped the samples into five clusters with a similarity level of 88.3% and a cluster distance ratio > 1, confirming the method's ability to distinguish kratom venations and other plant materials. This study demonstrates that FTIR-PCA is an effective, rapid, and non-destructive tool for profiling plant materials, although further research with a larger and more diverse sample set is needed for more robust predictive modeling.



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## 1. Introduction

Kratom (*Mitragyna speciosa*) is an endogenous Southeast Asia tree, that mainly grows in Thailand, Malaysia, and Indonesia[1], [2]. Kratom leaves is traditionally consumed to treat various ailments, as stimulants to sustain energy, and for its opioid-like analgesic and sedative effect[3], [4], [5], [6]. These effects made kratom gain popularity in Western countries, in particular for its analgesic activity[7]. Research has shown that both stimulant and sedative dose-dependent effects do exist, but a growing concern for its safety due to an increase in hospital visits and deaths that are related to its use made its legal status vary in some countries[8]. However, kratom products are still easily accessed through the Internet and remain an unregulated market in Western countries. Kratom is often marketed

based on the colors of its leaf vein, each with a different set of activities, although the total alkaloid compounds in not statistically different[9], [10]. Kratom's legal status in Indonesia remains uncertain, as the plant was legally cultivated and traded until 2008 when it was banned for use in processed food and later banned for traditional medicine and health supplements in 2016. However, in 2020 kratom was listed as a medicinal plant by Indonesia's Ministry of Agriculture[11], [12]. Indonesia's National Narcotic Agency (BNN) is set on listing kratom as a Schedule 1 controlled substance by 2024, while Indonesia's Ministry of Trade is set to regulate its export regulation, making the legal status remain unclear[13], [14]. Combined with the mostly unregulated market for kratom, consumers might be exposed to dangerous, adulterated products. Thus, a rapid and inexpensive method to authenticate kratom venations will be needed.

Attenuated Total Reflectance-Fourier-transform infrared (ATR-FTIR) spectroscopy is an effective instrument for analyzing chemical compounds as well as distinguishing plant material. ATR-FTIR presents a rapid, inexpensive, non-destructive method for providing information on the chemical compound present in the sample[15]. This made FTIR spectroscopy a powerful tool for analyzing variations between species, and even between specimens of the same species in the fields of medical, food, and agricultural science[16]. FTIR can be used to analyze samples on a plethora of matrices: cotton fiber, wood grain, plant extracts, drugs, biological tissue, and many more[15], [16], [17] One function of FTIR that has gained popularity is fingerprint analysis, especially in combination with chemometric techniques[18]. Chemometric techniques are valuable tools to interpret complex chemical data using mathematical and statistical methods[19]. One such technique is Principal Component Analysis (PCA) is a method to reduce dimensionality and improve interpretability of datasets, which could create a mathematical model to differentiate samples based on their chemical characteristics[20], [21]. However, research combining FTIR's ability to directly analyze samples within its matrix has been underutilized in pharmaceutical science. Thus, this study aims to develop a PCA model based on direct FTIR spectra data to profile and differentiate three different kratom venations and common alkaloid-containing plant materials. This research contributes novel insights into the reliability of FTIR-PCA as a rapid, inexpensive, and non-destructive screening tool for directly distinguishing plant materials in pharmaceutical research.

## 2. Method

### 2.1. Samples

Five products of three different venations of kratom leaf powder (green, red, and white) were purchased from sellers in Pontianak, West Kalimantan, Indonesia. Five roasted coffee and black tea leaves products were purchased from sellers in Yogyakarta. Coffee and black tea leaves samples were ground into powder.

### 2.2. FTIR Analysis

All ground samples were directly measured with FTIR using Thermo Nicolet iS10 FTIR spectrometer with Thermo Smart iTR diamond ATR based on a method by Arifah [22]. The absorbance value was performed from 400 cm-1 to 4000 cm-1, with a 0.964 cm-1 reading interval. The sample window was rinsed with acetone between usage. Every sample measurement was accompanied by background collection.

### 2.3. Data Analysis

FTIR spectra were subjected to hierarchical cluster analysis (HCA) and PCA using Minitab® 22 software version 22.1. Incremental cluster analysis was conducted beforehand to reduce computational load, data redundancy, and noise to gain fewer, more representative variables, allowing PCA to capture essential variation within the dataset[23]. PCA aims to reduce dataset dimensionality by simplifying complex datasets into smaller uncorrelated variables called principal components (PCs)[21]. After PCA, cluster analysis on observation was conducted to verify the cluster formed in PCA.

## 3. Results and Discussion

In this study, FTIR was used to obtain spectra data of three kratom venations along with tea leaves and coffee. Several prominent peaks in all spectra data indicate that direct FTIR analysis on

ground plant material still allowed the detection of specific functional groups contained. All samples showed high absorbance at 3200-2800, 1600-1200, and 1000-750 cm-1 Despite similarities in peaks between different sample groups, the distinct difference in intensity and peak wavenumbers could be observed. Coffee showed additional peaks at 3000, 1700, 1100, and 700 cm-1, and tea at 1700 and 1100 cm-1. However, it should be noted that these peaks were obtained from direct analysis of plant material, thus interference from other primary and secondary metabolites such as carbohydrates is likely. Each spectra contain 3475 data points and are subjected to cluster analysis on variables to reduce the number of variables in PCA.

Cluster analysis is a technique used to look for patterns within a dataset by clustering observations or variables into different groups. The aim is to classify objects in a cluster based on similarity. Objects within the same cluster have high similarity, and high dissimilarity between clusters. Because of the large volume of data points, the incremental cluster analysis is performed to reduce computational complexity, followed by a final cluster analysis of the compiled data[24]. Repeated clustering could risk reducing data similarity, so a target similarity level of 99% for each clustering performed is set. As a result, after conducting two rounds of cluster analysis on the same data, the overall similarity level is at 98%. Cluster analysis could reduce the number of variables from 3475 to 126 in the first round. By the end of the final round of cluster analysis, the variables were reduced to 48. This means the cluster analysis could reduce the number of variables by 98.6% while maintaining a 98% similarity level. This process should increase the interpretability of the dataset by clustering variables with similar patterns, thus identifying and clustering redundant information. Selecting representatives of these clusters ensures that PCA is applied to the most relevant data. Since PCA lacks the ability to differentiate between important and redundant data, pre-clustering would help to minimize noise from redundant variables, improving the quality of the PCs, and making the analysis more robust[23], [25], [26].

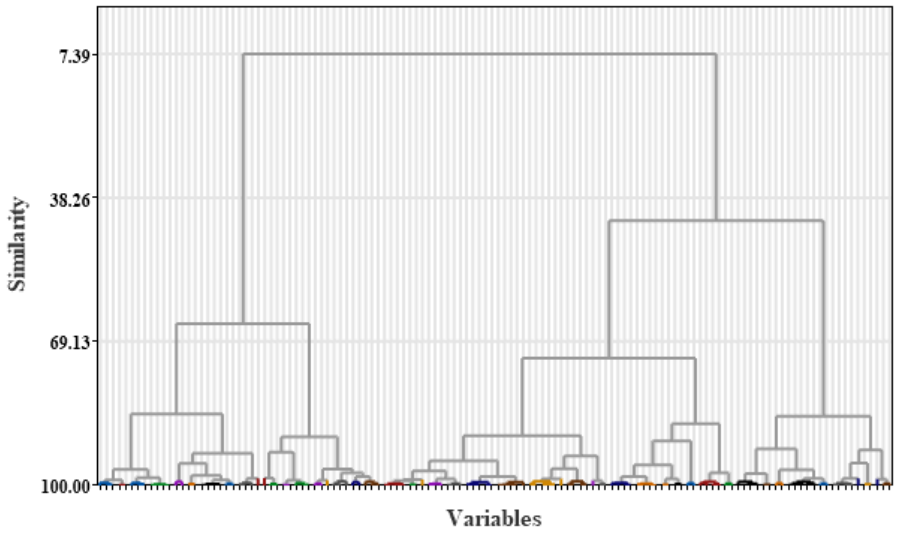


**Figure 1.** The cluster analysis of variables of 126 clusters from 7 incremental cluster analyses. The dataset is divided into 7 groups, and each group is subjected to cluster analysis with a 99% similarity level threshold.

The problem with dimensional reduction multivariate analysis is how to obtain a small number of variables that retain the most information available. One such method is PCA, which aims to reduce the variables of a dataset by creating a new variable called PC that is formed based on the total variance and the proportion of cumulates[27]. PCA focuses on retaining variance and capturing the most important patterns in the dataset. The impact of a PC could be measured by eigenvalue, representing the variance and significance of all the original variables captured. PC is considered significant when the eigenvalue >1, which from our data the significant PC are PC1, PC2, PC3, and PC4, representing 58,6 %, 35,5 %, 3,4%, and 2,4 % of the total variance respectively. PC1 was most impacted by peaks at 700-800 $cm^{-1}$, 1100-1200 $cm^{-1}$, and 2500 $cm^{-1}$. Meanwhile, PC2 was most impacted by peaks at 1700-1760 $cm^{-1}$. PC3 and PC4 were mainly impacted by 1990-2500 $cm^{-1}$ and 1760 $cm^{-1}$ respectively. PC1 and PC2 were selected due to having the best cumulative proportion of the variance in the dataset (93,9 %). Since the cumulative eigenvalue of the two PCs is > 70%, this means that PC1 and PC2 are

enough to describe the variance in the dataset[27]. The score plot showed that the method could differentiate the dataset into different clusters, indicating the effectiveness of the method in identifying kratom venations along with tea and coffee based on FTIR spectra of plant materials without further preparation. Differences within the same sample group could indicate variation due to source location, time of harvest, weather, and even preparation and storage method used[26], [28].

**Table 1.** Eigenvalue table of principal component analysis (PCA). Presented here are the PCs with eigenvalue > 1.

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| **Eigenvalue** | 28.111 | 16.959 | 1.654 | 1.164 |
| **Proportion** | 0.586 | 0.353 | 0.034 | 0.024 |
| **Cumulative** | 0.586 | 0.939 | 0.973 | 0.998 |

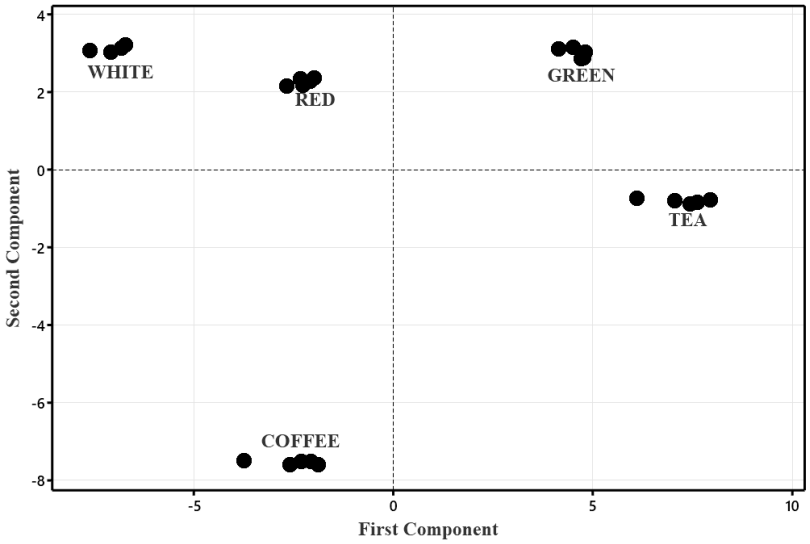


**Figure 2.** The PCA scoreplot of FTIR spectra data of five samples. All five sample groups are clustered separately.

Another cluster analysis was conducted with PC1 and PC2 values as variables to verify the partition for each cluster. The cluster analysis on observation was conducted to assess similarity across variables to verify PCA results. The cluster analysis could group samples into five clusters with a similarity level of 88,30%. Since a similarity level of 70% is commonly used as the threshold for significance, it could be said that the PCA model effectively identifies clusters that hold meaningful distinctions. Cluster analysis results showed that it needed 20 steps to group observation into five clusters. The final partition is able to group each sample correctly, indicating the desired separation is achieved. Evaluation of cluster separation shows that for each cluster the intra-cluster distance is ranged from 0,22 – 0,53, while the inter-cluster distance is ranged from 4.64 – 14.76. The small value of intra-cluster distance showed that the observations are tightly packed within clusters, indicating high similarity. The higher value of inter-cluster distance showed that the clusters are separated from each other, suggesting high dissimilarity. The combination of the two, using an inter-cluster to intra-cluster distance ratio, suggests that the clusters are significantly distinct as the ratio is far greater than 1[29]. From the cluster distance, we could infer that green kratom-tea and red kratom-white kratom may be more related than other combination.

**Table 2.** Distance inter-cluster and intra-cluster distance. Smaller distances within clusters indicate with high distance between clusters indicate distinct grouping.

| Sample | Inter-cluster | | | | | Intra-cluster |
|---|---|---|---|---|---|---|
| | ***Coffee*** | ***Tea*** | ***Green*** | ***Red*** | ***White*** | |
| **Coffee** | 0.0000 | 11.8481 | 12.7187 | 9.80634 | 11.5710 | 0.5267 |
| **Tea** | 11.8481 | 0.0000 | 4.6402 | 9.97954 | 14.7603 | 0.5269 |
| **Green** | 12.7187 | 4.6402 | 0.0000 | 6.89404 | 11.5843 | 0.2518 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Red** | 9.8063 | 9.9795 | 6.8940 | 0.00000 | 4.8087 | 0.2192 |
| **White** | 11.5710 | 14.7603 | 11.5843 | 4.80874 | 0.0000 | 0.2921 |

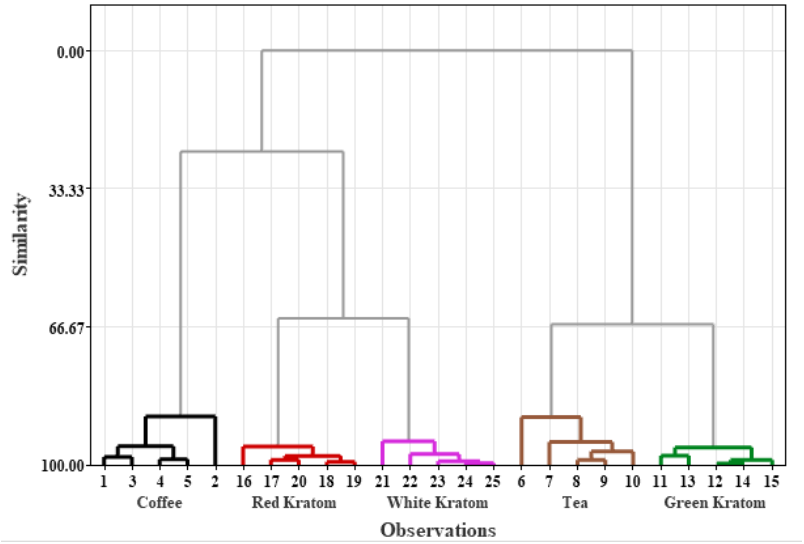


**Figure 3.** The cluster analysis of observations from PCA values. Note that the five clusters formed is able to group all five sample groups correctly.

Although the results of the PCA were satisfactory, the limited dataset posed constraints, particularly in the division into distinct training and testing sets for prediction. Expanding the sample size should address these limitations and enable more robust modeling, allowing the dataset to be split in a manner that supports reliable predictive analysis. Collecting additional samples with greater diversity should be proven beneficial to developing a better prediction model. This diversity is essential for accurately representing population variance and improving the model's applicability.

## 4. Conclusion

This study demonstrates the effectiveness of FTIR spectroscopy and subsequent PCA and cluster analysis, for directly characterizing and distinguishing plant materials based on their FTIR spectral data. The results confirm that FTIR can capture characteristic functional group information from ground plant samples. The initial cluster analysis effectively reduces the dimensionality of the variables, allowing a more focused PCA based on relevant variables. The PCA results, highlighting PC1 and PC2 as the main components, underscore the potential of this approach for differentiating between kratom venations and other plants, even with minimal sample preparation. The verification through cluster analysis of PC1 and PC2 provided validation of the sample partitioning, yielding five distinct clusters with an 88.3% similarity level. The intra-cluster to inter-cluster distance ratio indicates significant separation between clusters, emphasizing the specificity of the model. However, limitations due to the small sample size indicate the need for a larger, more diverse dataset to enhance model accuracy for a better predictive capability.

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## Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.