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FT-IR Characterization and Phylogenetic Analysis of Patchouli Oil from West Aceh and Aceh Jaya using PCA and HCA

KHAIRUN NISAK[1], SANDIANA NARISKA[1], KHAIRI SUHUD[1], ELLY SUFRIADI[1,2*]

[1]Chemistry Department, Faculty of Mathematic and Natural Science, Universitas Syiah Kuala, Banda Aceh, Indonesia
[2]Herbal Medicine Research Center, Universitas Syiah Kuala, Banda Aceh, Indonesia
*Corresponding Author

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Abstract. This study aims to characterize and compare the chemical composition of patchouli oil from various regions in Aceh, Indonesia, using Fourier-transform Infrared Spectroscopy (FT-IR) combined with Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Patchouli oil samples from five distinct locations within West Aceh and Aceh Jaya Regencies were analyzed, focusing on the 1800-500 cm^{-1} range. The FT-IR spectra revealed significant chemical markers, with notable absorption bands corresponding to aliphatic C-H, C=O, and C=C stretching vibrations. Multivariate analyses showed distinct clustering patterns, with four samples (POSM, POSP, POT, and POW) exhibiting high chemical similarity and clustering together, while one sample (POP) formed a separate cluster, indicating a lower degree of similarity. PCA analysis of the original spectra in the 1800-500 cm^{-1} range yielded a cumulative PC1 and PC2 value of 89%. The HCA dendrogram highlighted a significant relative distance of 10 between the POP sample and the other four samples, indicating minimal relatedness, with the closest relatedness observed between POSM and POSP, having a relative distance of 1.1. This study demonstrates the effectiveness of FT-IR spectroscopy combined with PCA and HCA in distinguishing chemical profiles of patchouli oil from different regions, providing insights into geographical and varietal influences on oil composition. The findings are crucial for quality control and standardization in the patchouli oil industry, with further molecular studies recommended to corroborate and enhance understanding of genetic diversity among patchouli varieties in Aceh.

Keywords: Patchouli oil, phylogenetic Analysis, , chemometrics, FT-IR characterization

INTRODUCTION

Patchouli, scientifically known as *Pogostemon cablin Benth*, belongs to the family Lamiaceae, class Magnoliopsida, and order Lamiales [1]. Patchouli oil is an essential oil derived from the aromatic herbaceous plant patchouli. This plant is native to subtropical regions of the Himalayas, Southeast Asia, East Asia, China, Brazil, Malaysia, and Indonesia. In Indonesia, several provinces such as Java, Kalimantan, Sulawesi, and Sumatra are major centers for patchouli production. Patchouli oil from Aceh Province is renowned for its superior quality, making Aceh the leading producer and helping Indonesia dominate the international market for high-quality patchouli oil [2], [3]. Indonesia's patchouli oil is a crucial export commodity, commanding 80-90% of the global market share. In 2017, the cultivated area for patchouli plants reached 20,508 hectares, with a production output of 2,207 tons. Sumatra has the largest cultivation area, covering 8,021 hectares and producing 1,243 tons of patchouli oil. Patchouli cultivation in Indonesia is typically carried out by individual farmers rather than state or private enterprises (Dirjenbun, 2019). Over time, countries like India, Singapore, Malaysia, China, Vietnam, and West Africa have also begun large-scale production of patchouli [4], [5]

Three types of patchouli are cultivated in Indonesia: Pogostemon hortensis, Pogostemon heyneanus, and Pogostemon cablin. P. hortensis is known as soap patchouli, P. heyneanus is referred to as Javanese patchouli, and P. cablin is commonly called Acehnese patchouli [6]. P. cablin is the most extensively cultivated species due to its high oil content and quality that meets commercial standards [7], [8]. Patchouli has various vernacular names: "nilam" in Indonesia, "patchouli" in English, *dhalum wangi* in Malaysia, *kabling* in the Philippines, *tamala patra* in India, and *pimsem* in Thailand[1], [9].

The production and composition of patchouli oil are influenced by several factors, including chemical properties, physical properties, and external influences such as climate and land characteristics, including altitude, slope gradient, and surface rock conditions. Variability within plant subspecies also affects the composition and amount of oil components (Aisyah et al., 2008). The main components of patchouli oil are patchouli alcohol and norpatchoulenol, with patchouli alcohol being the dominant compound contributing to the characteristic scent of the oil [10]. Patchouli alcohol determines the aroma of patchouli oil [11], known as "patchouli oil" in the international market[7].

Indonesia is the largest supplier of patchouli oil for international trade, with 60% of the supply coming from Aceh Province. Aceh is the leading producer in Indonesia, known for its high-quality oil [12]. West Aceh and Aceh Jaya are key patchouli-producing regions within Aceh Province. Initially, Aceh Jaya was part of West Aceh, with Meulaboh as the capital. In 2002, the Indonesian government passed Law No. 4/2002, leading to the formation of Aceh Jaya from West Aceh. Various districts in West Aceh and Aceh Jaya continue to cultivate patchouli, with samples obtained from Panga, Sampoiniet, and Teunom (Aceh Jaya) and Woyla and Sungai Mas (West Aceh). It is noted that farmers in these regions use different varieties of patchouli.

Three patchouli varieties are cultivated in Aceh: Lhokseumawe, Sidikalang, and Tapak Tuan. Previous studies have highlighted differences among these varieties in terms of morphology and oil quality. Due to the use of different varieties, maintaining consistent oil quality from West Aceh and Aceh Jaya is challenging. Buyers need unique and specific patchouli oil characteristics to match their end products, necessitating the identification of the main features of patchouli oil from specific regions (districts/subdistricts). Thus, it is essential to identify the chemical uniqueness and similarity of patchouli oil from these areas. This study also aims to explore the chemical similarities between patchouli oil from Aceh Jaya and West Aceh, providing insights into the phylogenetic relationships of patchouli plants in these regions despite using different varieties[3], [13], [14].

This study introduces several novel aspects that significantly advance the understanding of patchouli oil. By applying an integrated approach of FTIR spectroscopy combined with PCA and HCA, this research provides a comprehensive analysis of the chemical profiles of patchouli oil from different regions in Aceh, offering detailed insights into regional variations and their impacts on oil quality[15], [16]. Identifying unique chemical markers enhances the traceability and authenticity of the oil, crucial for maintaining Indonesia's competitive edge in the global market. Additionally, the study explores the phylogenetic relationships among patchouli varieties, elucidating genetic diversity and informing breeding programs for improved oil yield and quality. Addressing the gap in understanding the influence of geographical and varietal factors, this research provides a robust dataset that highlights the interplay between environmental conditions and plant genetics[17]. These contributions have practical implications for quality control, certification, and sustainable cultivation practices, ensuring the continued prominence of Indonesian patchouli oil in the global market.

METHODOLOGY

Materials

The materials used in this study include patchouli oil obtained from four different distillation sites and self-distilled dried patchouli plants, 90% and 95% alcohol, 0.1 N KOH solution, and phenolphthalein indicator solution. The samples utilized in this research consist of patchouli oil and dried patchouli plants. The patchouli oil was sourced from four distinct locations within West Aceh Regency and Aceh Jaya Regency, specifically Sungai Mas in West Aceh and Panga, Teunom, and Sampoiniet in Aceh Jaya. The dried patchouli plants originated from Woyla, West Aceh Regency. The sampling locations and geographic data of the sampling sites are presented in Figure 1 and Table 1 [7], [18].

The instruments employed in this study include Fourier-transform Infrared Spectroscopy (FTIR): Shimadzu IR-Prestige 21, Gas Chromatography-Mass Spectrometry (GC-MS): Shimadzu GCMS-QP2010plus, Unscrambler X 10.4.6 software from CAMO Analytics, and Origin Lab software. Each sample from the four locations was labeled for differentiation: MNSM for Sungai Mas, MNT for Teunom, MNP for Panga, MNSP for Sampoiniet, and MNW for Woyla.

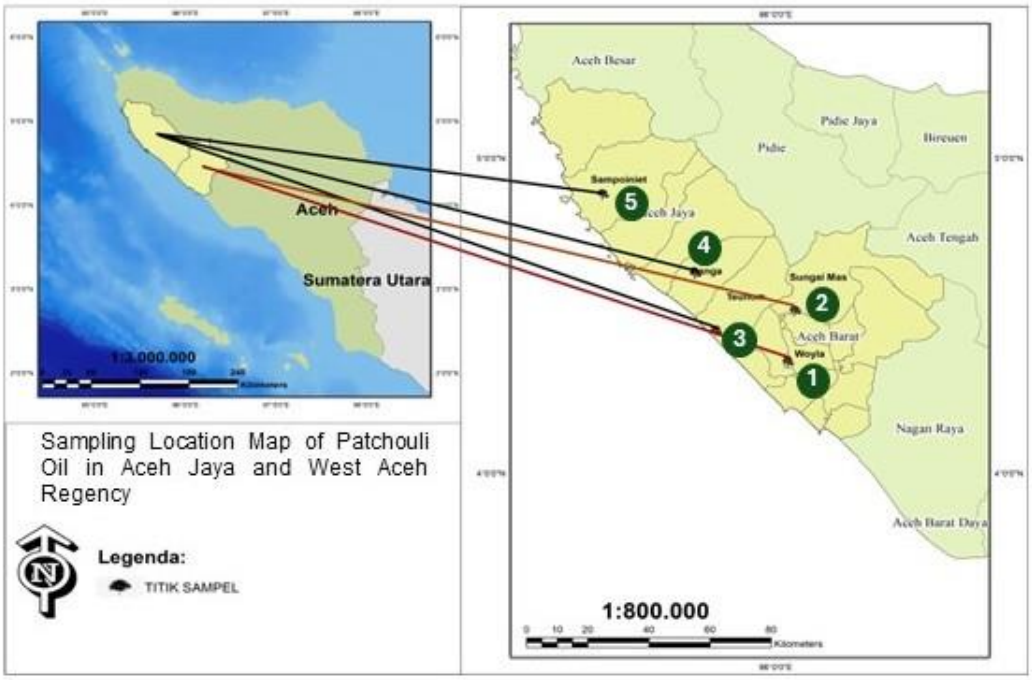


Figure 1. Sampling Location Map

Tabel 1. Geographical information of sampling locations

Point	District	Subdistrict	Sample code	Coordinate	Elevation (masl)
1	Aceh Barat	Woyla	POW	4°21'01.8"N 96°02'09".6"E	9
2		Sungai Mas	POSM	4°30'48.7"N 96°03'24".6"E	23
3	Aceh Jaya	Teunom	POT	4°27'02.1"N 95°49'29.3"E	7
4		Panga	POP	4°37'55.3"N 95°45'48.9"E	44
5		Sampoiniet	POSP	4°52'59.8"N 95°29'29".5"E	10

Procedure

Patchouli Oil Quality Testing
The quality of patchouli oil was assessed based on five parameters according to SNI 06-2325-2006: color, refractive index, solubility in 90% alcohol, acid number, and patchouli alcohol content.

Color Testing
A 10 mL sample was pipetted into a container and observed directly from a distance of 30 cm (Abraham et al., 2019).

Refractive Index Testing
The refractive index was measured using a refractometer at standard temperature. Patchouli oil was pipetted and dropped onto the refractometer prism, followed by reading the refractive index. The prism was cleaned with alcohol before and after use [19], [20].

Solubility in 90% Alcohol Testing
A 1 mL sample of patchouli oil was placed in a test tube and 90% alcohol was added dropwise until the solution became clear. The test was conducted at 20°C [5]

Acid Number Testing
A 4 g sample of patchouli oil was dissolved in 95% ethanol, followed by the addition of 3-5 drops of phenolphthalein indicator. The solution was titrated with 0.1 N KOH until a pink color appeared [21]

f. FT-IR Scanning of Patchouli Oil
Sample Preparation: Patchouli oil samples were collected from five different locations: Sungai Mas, Teunom, Panga, Sampoiniet, and Woyla. Each sample was labeled accordingly as MNSM, MNT, MNP, MNSP, and MNW. A 10 mL sample from each location was prepared for FT-IR analysis.

Scanning Process: The functional group characterization of patchouli oil samples was performed using Fourier-transform infrared (FT-IR) spectroscopy. The FT-IR analysis was conducted using a Shimadzu IR-Prestige 21 instrument. The spectral range for the analysis was set between 4000 cm^{-1} and 500 cm^{-1}.

Sample Loading: Approximately 10 mL of each patchouli oil sample was loaded into the FT-IR spectrometer.

Spectral Acquisition: The FT-IR spectrometer recorded the absorption spectra of the samples, focusing on identifying key functional groups based on the specific absorption peaks.
Repetition for Accuracy: Each sample was scanned five times to ensure the accuracy and reproducibility of the results. The spectra obtained from the FT-IR analysis were collected and stored for further analysis.

Data Analysis: The FT-IR spectra were analyzed to identify the functional groups present in the patchouli oil. The peak positions in the infrared spectra were examined to determine the specific functional groups according to standard spectral libraries and references [4]

g. Multivariate Analysis of FTIR Spectra

The FTIR spectra were analyzed using PCA and HCA with Unscrambler X 10.4.6 software. Prior to PCA analysis, data was pre-treated to reduce noise and improve quality. The pre-treatment methods included first derivative (FD), second derivative (SD), standard normal variate (SNV), and multiplicative scatter correction (MSC) [22], [23]. Pre-treatment was applied to the wavenumber ranges of 4000-1800 cm^{-1} and 1800-500 cm^{-1}.

First Derivative (FD): Pre-treatment was performed by selecting Task, then Transform, Savitzky Golay, 1st Derivative, and clicking OK.

Second Derivative (SD): Pre-treatment was performed by selecting Task, then Transform, Savitzky Golay, 2nd Derivative, and clicking OK.

Standard Normal Variate (SNV): Pre-treatment was performed by selecting Task, then Transform, SNV, and clicking OK.
Multiplicative Scatter Correction (MSC): Pre-treatment was performed by selecting Task, then Transform, MSC, and clicking OK.

After obtaining the best data from one of the pre-treatment methods, the analysis proceeded to PCA. Chemometric Analysis using Principal Component Analysis (PCA).

PCA, a multivariate analysis method, was employed to reduce data dimensionality. After smoothing the data with pre-treatment methods, PCA analysis was conducted by selecting Task, then Analyze, and Principal Component Analysis. The data were identified and separated into several components, with the output being a plot that differentiates data according to its components.

Chemometric Analysis using Hierarchical Cluster Analysis (HCA): HCA, another multivariate analysis method, was used to analyze the chemical similarity of objects. FTIR spectrum data were processed using Unscrambler X 10.4.6 software. The analysis was initiated by selecting Task, then Analyze, and Cluster Analysis. The data were identified for similarities and grouped into separate clusters, with the output being a dendrogram that displays the similarity of the input data.

RESULTS AND DISCUSSION

Scanning of Patchouli Oil Samples from Five Geographical Locations
Patchouli oil obtained from five locations in West Aceh and Aceh Jaya Regencies was characterized using FT-IR spectroscopy. FT-IR spectroscopy was employed to identify the functional groups present in the patchouli oil and to analyze its spectrum [24]–[26]. The spectra were recorded over a wavenumber range of 1800-500 cm^{-1} (Vasconcelos et al., 2015). The resulting FTIR spectra are presented in Figure 2, with wavenumbers plotted on the x-axis and absorbance on the y-axis.

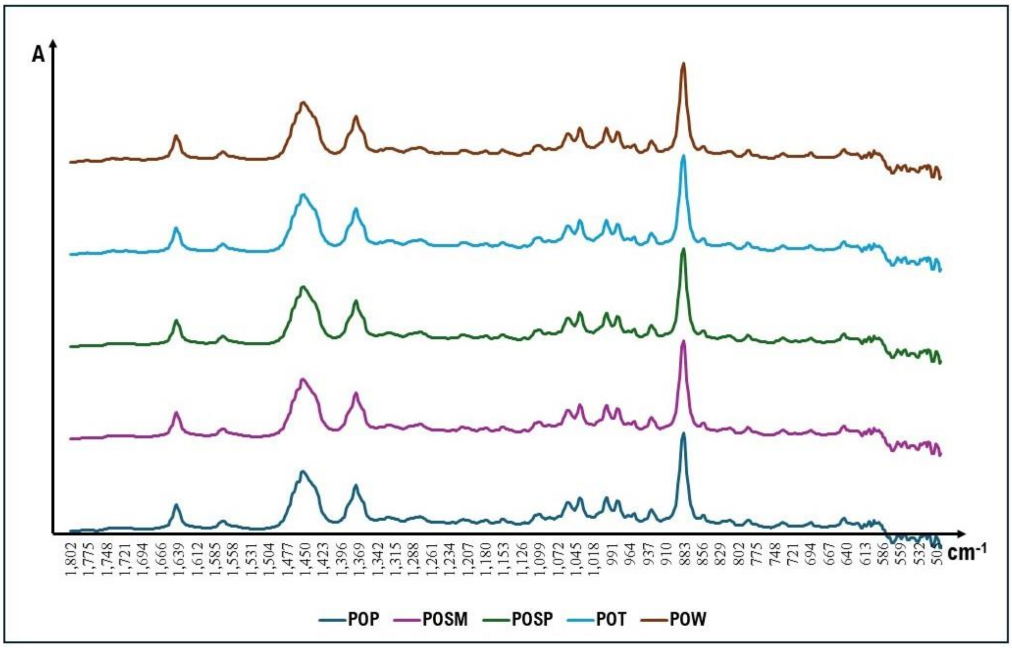


Figure 2. FT-IR spectra of patchouli oil from five different locations in Aceh Barat and Aceh Jaya regencies.

Patchouli oil obtained from five locations in West Aceh and Aceh Jaya Regencies was characterized using FT-IR spectroscopy. FT-IR spectroscopy was employed to identify the functional groups present in the patchouli oil and to analyze its spectrum [27]. The spectra were recorded over a wavenumber range of 3050-600 cm^{-1}. The resulting FTIR spectra are presented in Figure 2, with wavenumbers plotted on the x-axis and transmittance on the y-axis.

The FT-IR spectral analysis of patchouli oil samples from Aceh Jaya and Aceh Barat reveals significant insights into the chemical composition and functional groups present in the oils. The analysis focused on the absorption regions within the 1800-500 cm^{-1} range, which are critical for identifying key functional groups in the oil.

The absorption bands observed at 2850-2960 cm^{-1} indicate the presence of aliphatic C-H stretching vibrations. These bands are prominent in both Aceh Jaya and Aceh Barat samples, demonstrating a strong indication of aliphatic chains. This suggests a high content of aliphatic hydrocarbons, which are typical constituents in essential oils and contribute to the overall hydrophobic character of the patchouli oil[28]–[30].

The absorption region at 1650-1750 cm^{-1} corresponds to the C=O stretching vibrations, indicative of carbonyl groups. The significant presence of these bands in both Aceh Jaya and Aceh Barat oils points to the existence of ketones and aldehydes, which are vital components that contribute to the aromatic and therapeutic properties of patchouli oil. The carbonyl stretching indicates the structural complexity and the potential diversity of the oxygenated compounds within the oil.

The absorption bands in the 1500-1600 cm^{-1} range are attributed to C=C stretching vibrations, signifying the presence of aromatic or alkenic compounds. Both oil samples exhibit these bands, indicating a sufficient quantity of such compounds. Aromatic compounds are crucial for the fragrance profile of the oil, while alkenes may play a role in its reactivity and stability.

The 1375-1450 cm^{-1} region, associated with CH_3 bending vibrations, reveals the presence of clearly defined methyl groups in both patchouli oils. The consistent occurrence of these bands highlights the contribution of methyl-substituted aliphatic chains to the overall molecular structure of the oils. These groups are essential for the hydrophobic properties and influence the volatility and scent profile of the patchouli oil.

The absorption observed in the 700-900 cm^{-1} range, corresponding to C-H out-of-plane bending vibrations, further substantiates the presence of alkenic or aromatic structures. These bands are evident in both Aceh Jaya and Aceh Barat samples, underscoring the structural diversity and complexity of the patchouli oil. The aromatic rings and alkenes contribute significantly to the oil's stability and reactivity, enhancing its utility in various applications.

The FT-IR spectra of patchouli oils from Aceh Jaya and Aceh Barat within the 1800-500 cm^{-1} range reveal a rich tapestry of functional groups that define their chemical profiles. The strong indications of aliphatic chains, significant presence of carbonyl groups, and the sufficient quantity of aromatic or alkenic compounds underscore the high quality and complexity of the oils. The clearly defined methyl groups and evident alkenic or aromatic structures further highlight the oils' distinctive characteristics. While the oils from both regions share these key features, the slight variations in absorption intensity may reflect differences in environmental conditions, cultivation practices, and genetic factors, contributing to the unique profiles of patchouli oil from these regions[25], [31].

This comparative analysis underscores the importance of FT-IR spectroscopy in elucidating the detailed chemical composition of essential oils, facilitating the identification of geographical and botanical influences on their quality and characteristics. Such insights are invaluable for ensuring the standardization and optimal utilization of patchouli oil in various industrial and therapeutic applications.

The transmittance results from FTIR characterization were further processed using chemometric methods, specifically PCA and HCA, to obtain a more detailed analysis.

Multivariate Analysis of FTIR Spectra

The absorbance results from FTIR characterization were processed using the chemometric software Unscrambler X 10.4.6. The chemometric analyses employed were PCA and HCA. Before performing PCA, data underwent pre-treatment. Pre-treatment serves to smooth the data and reduce noise, thereby stabilizing the data without losing essential information ([32], [33]. Four pre-treatment methods were utilized: First Derivative (FD), Second Derivative (SD), Standard Normal Variate (SNV), and Multiplicative Scatter Correction (MSC) [23], [34]. Pre-treatment was conducted over wavenumber ranges 4000-500 cm^{-1} and 1800-500 cm^{-1}. The results of the data processing using the four pre-treatments are shown in Table 2.

Table 2. Pattern recognition analysis results via pca on original and pre-processed spectra

No.	Spectra source	PC1:PC2 (4000-500 cm^{-1})	PC1:PC2 (1800-500 cm^{-1})
1.	Original	77%:11%	81%:9%
2.	FD	54%:16%	49%:25%
3.	SD	50%:12%	52%:12%
4.	SNV	46%:12%	44%:21%
5.	MSC	46%:11%	43%:21%

Based on the table above, we observe that the best values were obtained from the original spectra in the wavenumber range of 1800-500 cm^-1, with the cumulative value of PC1 and PC2 reaching 89%. In the wavenumber range of 4000-500 cm^-1 from the original spectra, the results were also very good, with the cumulative PC1 and PC2 reaching 88%. Therefore, subsequent analyses will utilize both of these original spectra ranges.

In the study of patchouli oil, it is often observed that pre-processing does not necessarily improve the analysis[35]. This study also shows that treatment of the original spectra did not enhance the interpretative results, as evident in **Table 2** and **Figure 3**. This indicates that the original spectral data provides a more accurate representation of the sample's chemical composition, thereby making it more reliable for further analysis[36].

The implication is that, for patchouli oil, maintaining the integrity of the original spectra is crucial for obtaining accurate and meaningful analytical results. This reinforces the notion that while pre-processing techniques can be beneficial in many spectroscopic analyses, they are not universally applicable and must be evaluated on a case-by-case basis[37].

These findings highlight the importance of careful consideration of pre-processing steps in FT-IR spectroscopy, particularly in essential oil analysis, where the preservation of original spectral features is critical for accurate classification and quality control[38].

PCA Analysis

Principal Component Analysis (PCA) can effectively distinguish sample groups based on principal components and chemical similarities (Rismawati, 2018; Yuliani et al., 2018). The PCA results for the five samples from Aceh Barat and Aceh Jaya are illustrated by the score plot in Figure 4.4. It is evident that there are four distinct quadrants that differentiate the data grouping. The PCA analysis results show that four samples are located in the same quadrant, while one sample is positioned in a different quadrant. This results in varying distances between each sample, indicating the degree of similarity among them. The greater the distance between samples, the lower the similarity they share [39], [40].

The PCA score plot demonstrates that the POP sample exhibits minimal similarity with the other four samples. This is evidenced by the POP sample's position in a different and distant quadrant compared to the others. In contrast, the four other samples—POSM, POSP, POT, and POW—display closer similarities to each other. Specifically, MNSP shows a higher degree of similarity to POSM due to their proximity, despite being in different quadrants. The POT sample is similar to POSP as they are in the same quadrant, although the distance between POSP and POT is slightly greater than between POSP and POSM. MNW is similar to POSM because they share the same quadrant, but the distance is greater compared to POSM-POSP[39].

The PCA score plot cannot conclusively determine whether POT or POW is closer to the POSM-POSP group. Therefore, further analysis using Hierarchical Cluster Analysis (HCA) is necessary to elucidate these relationships[41].

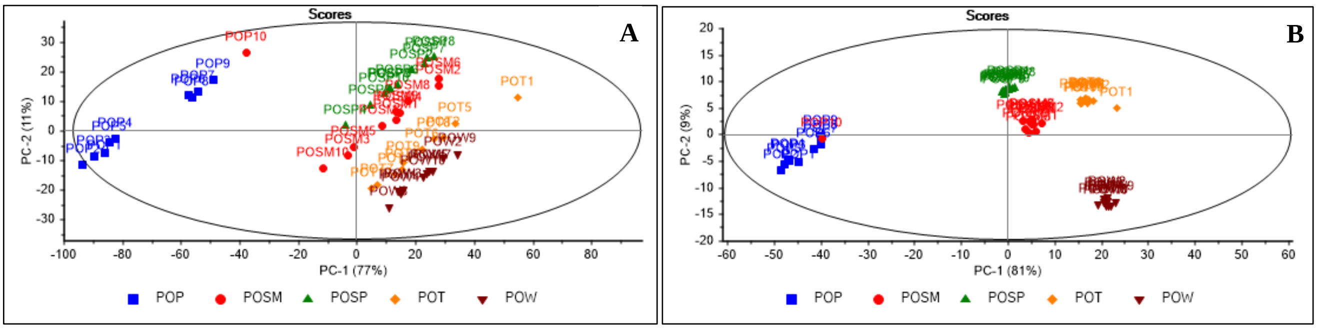


Figure 3. Comparison of PCA score plots for patchouli oil from Aceh Barat and Aceh Jaya using spectra in the wavenumber range 4000-500 cm^{-1} (A) and 1800-500 cm^{-1} (B)

Hierarchical Cluster Analysis (HCA)

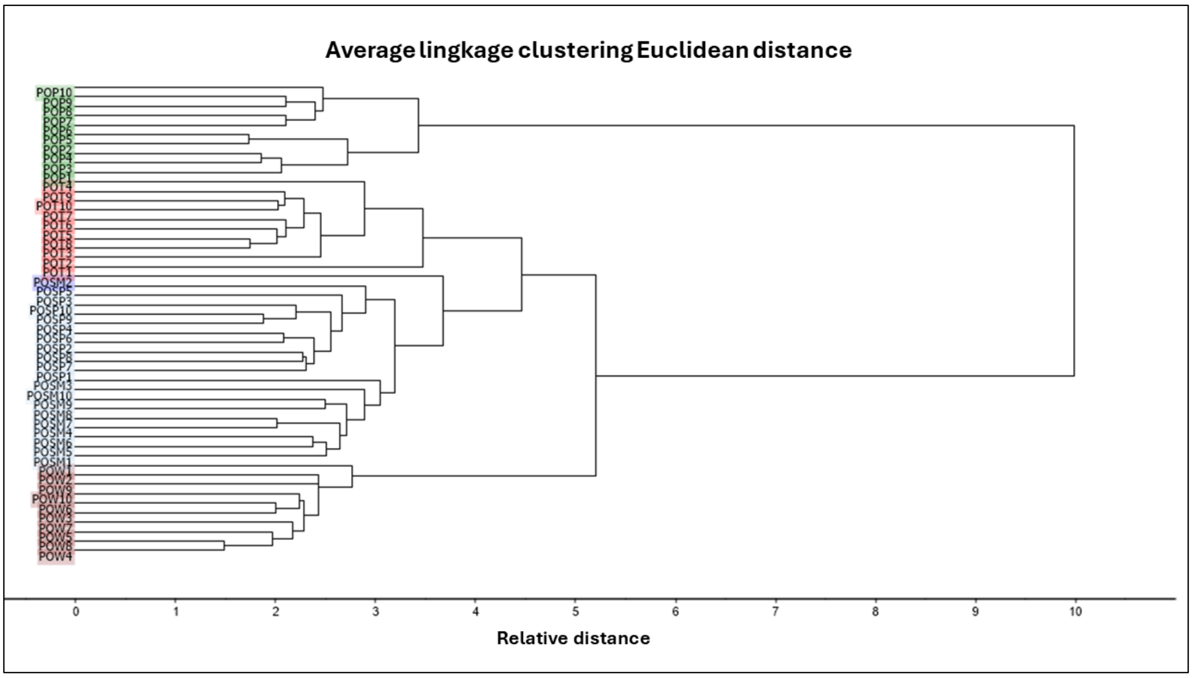
Hierarchical Cluster Analysis (HCA) is a robust technique used to categorize data into distinct hierarchical clusters, each representing varying degrees of relatedness among the samples (Yang et al., 2016). The HCA results, depicted by the dendrogram in Figure 4.6, illustrate two major clusters separating the data from the five analyzed patchouli oil samples. Cluster 1 comprises the POP sample, sourced from Panga subdistrict, Aceh Jaya, while Cluster 2 includes the POSP sample (from Sampoiniet subdistrict, Aceh Jaya), POSM sample (from Sungai Mas subdistrict, Aceh Barat), POW sample (from Woyla subdistrict, Aceh Barat), and POT sample (from Teunom subdistrict, Aceh Jaya)[42].

The dendrogram clearly indicates that the POP sample does not share a close relatedness with the oils from the other four locations. The convergence of these two clusters at a relative distance of 10 suggests minimal relatedness between them. In hierarchical clustering, a distance nearing the relative value of 0 signifies a very close relationship among samples, whereas an increasing distance from this value indicates a decreasing degree of relatedness [43]. Therefore, the substantial relative distance of 10 between Cluster 1 and Cluster 2 confirms the lack of close relatedness between these clusters (**Figure 4**).

Within Cluster 2, comprising samples POT, POW, POSM, and POSP, varying degrees of relatedness are evident, forming three sub-clusters with the largest relative distance being 3.3, observed between the POT cluster and the other three samples. The POW cluster, which groups with two other samples, has a distance of 2.2, while the POSP and POSM cluster shows a relative distance of 1.1. This indicates that POSP and POSM share the closest relatedness, followed by POW and POT (**Figure 4**). (**Figure 4**). The samples POSM, POSP, POT, and POW demonstrate mutual relatedness, supporting the hypothesis that they may originate from the same patchouli variety.

The differences in relatedness, particularly the distinct clustering of the POP sample, are hypothesized to arise from unique components identified in the POP sample, as suggested by the GCMS results. This divergence suggests that POP may belong to a different patchouli variety than POSM, POSP, POT, and POW. Aceh is known for cultivating three main patchouli varieties: Tapaktuan, Lhokseumawe, and Sidikalang [40], [44]. Given the possibility that farmers might not be fully aware of the specific varieties they cultivate, mixed cultivation is likely, leading to the observed clustering patterns.

This phylogenetic analysis through HCA provides crucial insights into the genetic and chemical diversity among patchouli oil samples from different regions. The distinct clustering of POP suggests a potential varietal difference, underscoring the importance of accurate varietal identification and cultivation practices. Further research, potentially integrating molecular techniques, could confirm these findings and provide a more detailed understanding of the genetic diversity and relationships among patchouli varieties in Aceh. This knowledge is essential for optimizing cultivation strategies, ensuring the consistency of oil quality, and preserving the genetic diversity of patchouli plants[45]–[47].



CONCLUSION

This study successfully characterized the chemical composition of patchouli oil from five locations in Aceh Jaya and West Aceh using FTIR spectroscopy and chemometric methods. The FTIR analysis identified key functional groups, including aliphatic chains, carbonyl compounds, and aromatic structures, which are critical to the oil's quality. PCA results demonstrated distinct groupings of the oil samples, indicating varying degrees of chemical similarity. HCA further revealed two primary clusters, suggesting potential varietal differences, particularly highlighting the unique profile of the sample from Panga subdistrict. These differences are hypothesized to stem from genetic variation and environmental influences. The findings emphasize the importance of maintaining the integrity of original spectral data for accurate chemical analysis and the need for rigorous quality control in patchouli oil production. This study contributes valuable insights into the phylogenetic relationships and chemical profiles of patchouli oils, enhancing their standardization and utilization in diverse applications.

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