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Identification of Several Fractions of Patchouli Alcohol from Patchouli Oil (*Pogostemon cablin* Benth.) using Combination Method of Infrared Spectroscopy and Principal Component Analysis

(Pengenalpastian Beberapa Fraksi Alkohol Nilam daripada Minyak Nilam (*Pogostemon cablin* Benth.) menggunakan Kaedah Gabungan Spektroskopi Inframerah dan Analisis Prinsip Komponen)

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ABSTRACT

Patchouli oil is a type of essential oil that is very popular all over the world because it is rich in benefits and has high economic value as well. The quality of it must be maintained and strictly controlled to prevent counterfeiting. In this study, the patchouli oil sample used was Aceh patchouli oil of the Tapak Tuan variety, Indonesia. The patchouli oil was redistilled and crystallized to obtain 5 varying levels of patchouli alcohol. This study aims to evaluate the application of chemometrics and Fourier transforms infrared (FTIR) spectroscopy as simple, effective and economical methods to assess the quality of patchouli oil and patchouli crystals. FTIR data from the 5 samples were subjected to multivariate analysis consisting of Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). HCA, PCA and LDA managed to distribute the tested samples into informative groups. The results of the HCA analysis showed the pattern of sample classification based on patchouli oil concentration, the results of the PCA analysis showed that the PCA plot scores were quite adequate in distinguishing patchouli oil concentrations. The results of the LDA analysis showed the authenticity of the patchouli oil spectrum based on the results of PCA analysis. From the cross-validation accomplished, this method was successful in predicting accurately the concentration of patchouli oil samples. The results of the analysis concluded that the combination of the FTIR-Chemometric method is a fast, easy, accurate and inexpensive method. In addition, this analysis can be executed without damaging the sample.

Keywords: Infrared spectroscopy; patchouli alcohol; patchouli crystal; patchouli oil

ABSTRAK

Minyak nilam adalah sejenis minyak pati yang sangat popular di seluruh dunia kerana ia kaya dengan manfaat dan mempunyai nilai ekonomi yang tinggi. Kualitinya mesti dikekalkan dan dikawal ketat untuk mengelakkan pemalsuan. Dalam kajian ini, sampel minyak nilam yang digunakan ialah minyak nilam Aceh varieti Tapak Tuan, Indonesia. Minyak nilam telah disuling semula dan dihablurkan untuk mendapatkan 5 tahap alkohol nilam yang berbeza-beza. Kajian ini bertujuan untuk menilai aplikasi kemometrik dan spektroskopi transformasi Fourier inframerah (FTIR) sebagai kaedah yang mudah, berkesan dan menjimatkan untuk menilai kualiti minyak nilam dan hablur nilam. Data FTIR daripada 5 sampel telah tertakluk kepada analisis multivariat yang terdiri daripada Analisis Kelompok Hierarki (HCA), Analisis Prinsip Komponen (PCA) dan Analisis Diskriminasi Linear (LDA). HCA, PCA dan LDA berjaya mengedarkan sampel yang diuji ke dalam kumpulan bermaklumat. Hasil analisis HCA menunjukkan corak pengelasan sampel berdasarkan kepekatan minyak nilam, hasil analisis PCA menunjukkan skor plot PCA agak mencukupi dalam membezakan kepekatan minyak nilam. Hasil analisis LDA menunjukkan ketulenan spektrum minyak nilam berdasarkan hasil analisis PCA. Daripada pengesahan silang yang dicapai, kaedah ini berjaya meramal dengan tepat kepekatan sampel minyak nilam. Hasil analisis merumuskan bahawa gabungan kaedah FTIR-Kemometrik adalah kaedah yang cepat, mudah, tepat dan murah. Di samping itu, analisis ini boleh dilaksanakan tanpa merosakkan sampel.

Kata kunci: Alkohol nilam; kristal nilam; minyak nilam; spektroskopi inframerah

Introduction

Pogostemon cablin (Blanco) Benth (patchouli) is a plant belonging to the Lamiaceae family (Chen et al. 2020; Ito et al. 2016). Patchouli is a type of essential plant where the extraction of dried patchouli leaves and stems will produce an essential oil known as patchouli oil *(*patchouli oil) (He et al. 2016; Kusuma & Mahfud 2017; Ramya et al. 2013).

Patchouli oil (PO) is an important commodity that has been in use since antiquity (Beek & Joulain 2017; Das 2016; Wang et al. 2018). Because of its long-lasting scent and fixative capabilities, the oil is often used in the creation of perfumery items such as soaps, cosmetics, and detergents (Ermaya et al. 2019). Besides, research in pharmaceutical field has shown that the plant have anti-inflammatory effects (Zhao et al. 2020), anti-influenza (Yu et al. 2019), anti-tumor (Jeong et al. 2013), and as an antidepressant (Cahyono et al. 2019; Pujiarti et al. 2012).

Patchouli oil is Indonesia's most important agro-industrial export commodity. Indonesian patchouli oil controls about 80-90% of the international market, where the one from Aceh Province is a flagship export commodity, popular among American and European markets owing to its quality (Beek & Joulain 2017; Manglani & Deshmukh 2011). PO Aceh, especially the Tapak Tuan (South Aceh) variety, is called the best variety in the world because it contains a lot of patchouli alcohol chemical compounds (Ambrose et al. 2016; Nuryani 2006).

Patchouli alcohol (PA) is one of the chemical compounds contained in patchouli oil. PA is the primary chemical responsible for the quality (Asnawi et al. 2018) and its aroma (Donelian et al. 2009). PA acts as a fragrance binder to give long-lasting characteristics for the fragrance as compared to -guaiene, -guaiene, seychellene, or -patchoulol (Aisyah & Anwar 2012). Hence, if PA is present in greater levels, the market value of this oil may rise (Cahyono et al. 2019; Hu et al. 2017). Several studies have succeeded in creating patchouli crystals using PO as the raw material to increase the patchouli alcohol content (Rahmadina et al. 2020; Su et al. 2014).

To assess the authenticity and control the quality of patchouli oil and crystals, sophisticated analytical techniques and methods are needed that are fast, easy, accurate, and cost-effective (Kharbach et al. 2020). Fourier Transform Infrared (FTIR) Spectroscopy and Chemometrics are two of the most frequently used non-destructive analytical methods in evaluating plant chemical profiles (Yi et al. 2013) because they are considered more time-saving and environmentally friendly (Xu et al. 2015). The combination of FTIR and chemometrics has been used for various purposes, including to identify differences in the location of origin of a sample (Akbar et al. 2021; Wadood et al. 2019), to identify falsification (Guo et al. 2018), and is also used in forensic analysis (Kumar & Sharma 2018).

One of the statistical methods that can be used to analyze data from infrared spectroscopy measurements is Principal Component Analysis (PCA) (Karma et al. 2021). PCA is a statistical method that aims to reduce data from a complex data set. The use of Infrared-PCA combination has been widely used for identification and authentication of various samples. The results obtained indicate that the procedure can run well, powerful and simple to distinguish coffee leaves collected from different climatic conditions (Sanchez et al. 2018). On other published reports, the use of PCA could yield a good result in distinguishing seven types of patchouli oil (Diego et al. 2018). In addition, Wadood et al. (2019) suggested that NIR spectroscopy combined with chemometrics (PCA, LDA) could identify geographic differences, production years and genotypic classification of wheat plants. Previously, chemometric approach has been reported capable of differentiating the types of woods (Smith et al. 2019), identifying the types of woods (Sharma et al. 2020), and distinguishing herbal-based facial creams from non-herbal-based ones (Sharma et al. 2021).

The use of chemometric could save the cost for analysis due to its non-destructive nature, where previous published literatures had confirmed the non-destructive properties of PCA and its ability to differentiate fabrics from tree barks (Smith et al. 2019). This non-destructive approach had also been employed on chemometric investigation to differentiate herbal and non-herbal-based facial creams (Sharma et al. 2021). Another study had also reported the short-time and non-destructive process of wood identification using ATR-FTIR analytical technique in combination with chemometric methods (Sharma et al. 2020).

Several previous studies have tested the purity and quality of patchouli oil, yet there are still very few literatures discussing about testing techniques which are effective, simple and cost-effective. This research was conducted by combining several simple spectroscopic methods, especially FTIR-Chemometrics to see patchouli based on several percentage variations of patchouli alcohol. This study aims to control the quality of patchouli oil and crystals.

Materials and Methods

SAMPLE COLLECTION

The sample is Aceh patchouli oil from patchouli farmers in Alur Dua Mas Village, Tapak Tuan (South Aceh Regency, Indonesia) (Figure 1). The GCMS results showed that the patchouli oil had a patchouli alcohol content of PA 28.68%.

The patchouli oil sample was then re-distilled to obtain patchouli oil with PA content of 42.81%, 49.29%, and 60.66%. Some of the PO was crystallized to obtain patchouli crystals with 100% PA. The redistillation and crystallization processes were carried out at *the Atsiri Research Center* (ARC) Syiah Kuala University, Aceh, Banda Aceh, Indonesia. Table 1 and Figure 2 show the sample preparation of patchouli oil used in this study.

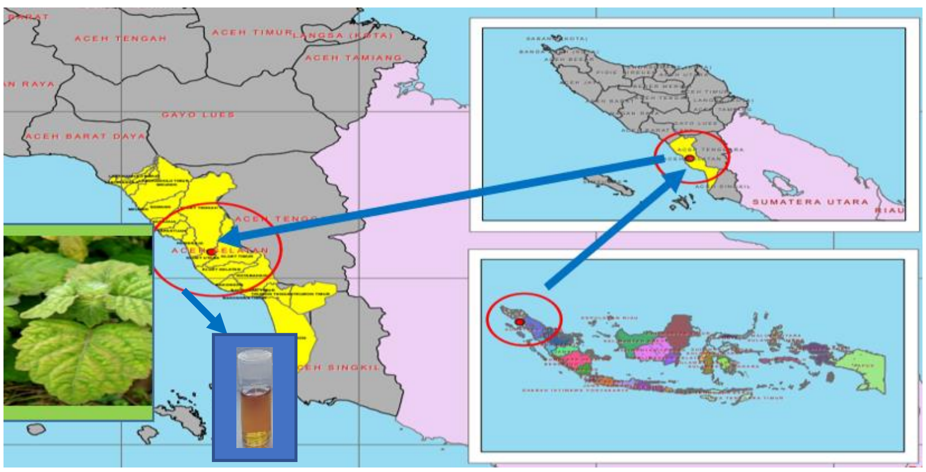

FIGURE 1. Location of origin of patchouli oil samples

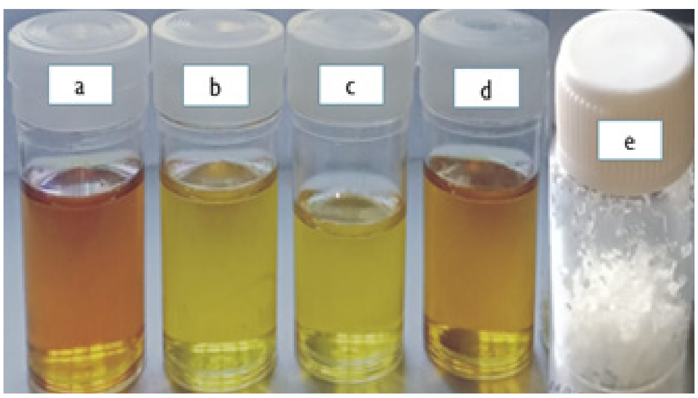

FIGURE 2. Aceh patchouli oil sample. a) traditional distillation of patchouli farmers (PA 28.68%), b) redistilled patchouli oil 1 (PA 42.81%), c) redistilled patchouli oil 2 (PA 49.29%), patchouli oil redistilled 3 (PA 60.66%), and Patchouli crystals (PA 100%)

TABLE 1. Variation of concentration/fraction of patchouli oil samples

No	Concentration/Fraction	Sample code	Form
A	100 %	PA-100%	Solid/Crystal
B	60,66 %	PA-60,66 %	Liquid
C	49,29 %	PA-49,29 %	Liquid
D	42.81 %	PA-42.81 %	Liquid
E	28.68 %	PA-28.68 %	Liquid

SAMPLE PREPARATION

Every 2 mL sample was put into a glass bottle and labeled.

THE ACQUISITION OF THE FTIR SPECTRUM

Acquisition of the FTIR spectrum was conducted at the Environmental Quality Testing Engineering Laboratory, Department of Chemical Engineering, Universitas Syiah Kuala. FTIR spectral analysis was performed using the Shimadzu (Japan) *IR Prestige* instrument. The sample analyzed by FTIR was patchouli oil with known alcohol content. The total FTIR spectrum of the 5 patchouli oil samples is 15 spectra. Samples were measured at a wavelength of 4000 - 400 cm^{-1} (Karma et al. 2021). The spectrum data obtained were then stored in excel format (xlsx) for further analysis.

CHEMOMETRIC ANALYSIS

The initial stage of chemometric analysis included data preprocessing, where the spectral transmittance was smoothed with Savitzky-Golay method. After being preprocessed, the data were analyzed using chemometric methods, *viz*. Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA), and Linear Discriminant Analysis (LDA). HCA and PCA were performed on Unscrambler ® 10.4 (CAMO SA, Oslo, Norwegian), whilst the LDA analysis - on Python 3.8.

RESULTS AND DISCUSSION

FTIR SPECTRA OF PATCHOULI OIL AND PATCHOULI CRYSTALS

Patchouli oil and crystals samples, which their spectrum acquired with the FTIR instrument, have been known the content level of their patchouli alcohol. FTIR measurement was carried out at a wavelength of 4000-400 cm^{-1}. Patchouli oil FTIR spectrum obtained is shown in Figure 3(a).

From the Infrared spectrum, it can be seen that the absorbance at wavenumber 3300 - 3500 cm^{-1} indicates the presence of the O-H functional group, then the presence of the Aromatic = C-C functional group at wavenumber 3100-3000 cm^{-1}, the aliphatic C-H functional group, the C=O functional group at 1740-1700 cm^{-1}, alkene C=C functional group at 1680-1600 cm^{-1}, aromatic C=C at wavenumber 1600-1475 cm^{-1}, $-CH_3$ group at wavenumber 1400-1325 cm^{-1} and C-O functional group at wavenumber 1100-1000 cm^{-1}.

PREPROCESSING DATA

Preprocessing data is an important step in chemometric analysis. In principle, not all the data must have a pre-treatment step, but under certain conditions preprocessing greatly affects the results, and this process was carried out to obtain the maximum results. In this study, the spectral spectra of patchouli oil and patchouli crystals were processed using smoothing method. This method served to reduce the bias effect of light scattering during the spectrum acquisition process (shown in Figure 3(b)).

CHEMOMETRIC ANALYSIS

The analysis of PA content in both patchouli oil and crystals has been conducted using GCMS. Unfortunately, this method requires expensive costs, which is very inefficient to be applied directly in the field. Currently, the combination of FTIR-Chemometry is a good choice to identify patchouli oil and crystals with different PA content. The chemometric methods used in this study were HCA and PCA (Cebi et al. 2017). In this study, characterization of the different oil content and patchouli crystals was carried out based on their FTIR spectrum.

HIERARCHICAL CLUSTER ANALYSIS (HCA)

HCA is also known as cluster analysis. This analysis is a type of unsupervised pattern recognition method which is often used by researchers to classify samples, and identify similarities between the analyzed samples (Cebi et al. 2017).

Based on the dendrogram in Figure 4, 5 groups were formed according to the concentration of patchouli oil and crystal, namely cluster 1 (sample of patchouli crystal with 100% PA level), cluster 2 (sample of patchouli oil with 28.68% PA level), cluster 3 (sample of patchouli oil with 49.29% PA level), cluster 4 (sample of patchouli oil with 42.81% PA level), and cluster 5 (samples of patchouli oil with 60.66% PA level). In general, by using the HCA analysis technique, the classification of patchouli oil and crystal has already been seen. However, the classification formed is not completely accurate yet, because if the cut line is drawn at a higher position, then, the sample grouping will be difficult to define. It is because some of the samples with significantly different concentrations will be in a group. In this study, HCA result provides information that clustering pattern can be defined, so that it will be characterized using PCA later.

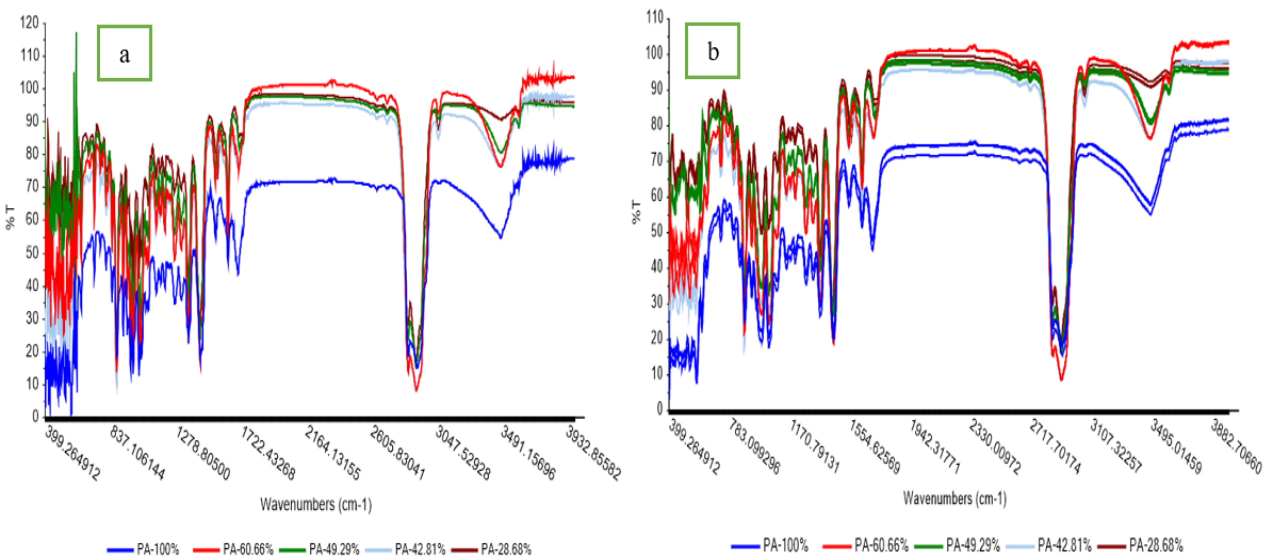

FIGURE 3. a) FTIR spectrum of sapphire oil and crystal, b) Spectrum of sapphire oil and crystal samples with smoothing pretreatment

The use of the HCA-FTIR technique has also been applied by Cebi et al. (2021) in order to analyze *Rosa damascene* essential oil. The result of the analysis shows that the HCA dendrogram specifically provides a visual presentation of the intrinsic relationship between the original sample and the redistilled one through the main cluster and sub-clusters. Another study stated that HCA results could distinguish concentrated essential oil samples extracted from the *Lamiaceae* (Kustrin et al. 2020).

PRINCIPAL COMPONENT ANALYSIS (PCA)

The aim of PCA was to classify the patchouli oil and patchouli alcohol crystal with different concentrations. The PCA is a technique of non-supervision pattern recognition which could be used to reduce the data and obtaining information to identify factors associated with the tendency of data grouping (Gad et al. 2013).

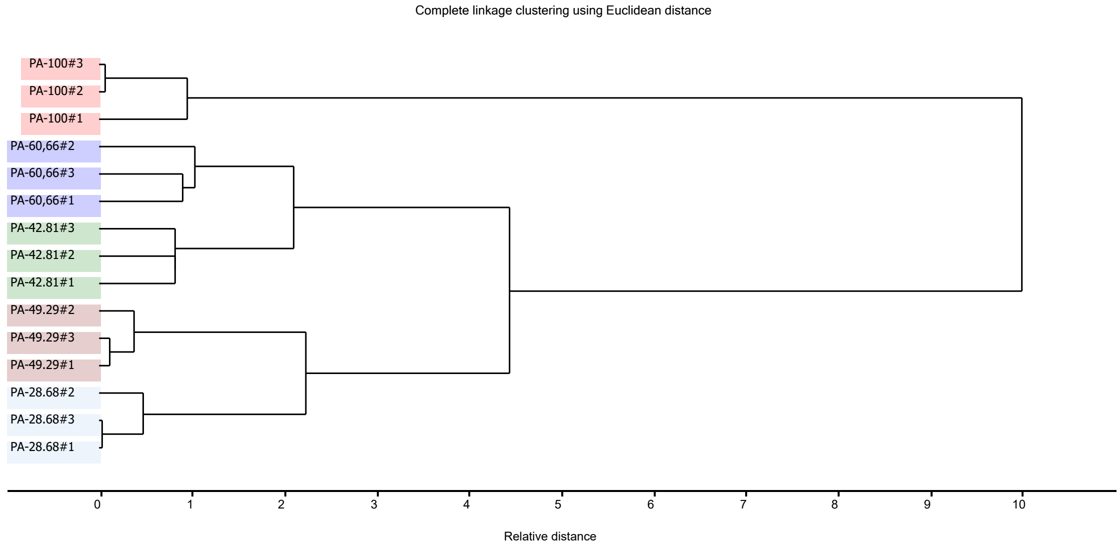

FIGURE 4. The results of oil and patchouli crystal's cluster analysis with FTIR-HCA

The combination of FTIR-chemometric previously had been employed to identify and authenticate a variety of samples, of which was coffee leaves collected from several locations with different climate conditions analyzed based on FTIR spectra and followed by PCA method (Sanchez et al. 2018). The results showed that FTIR spectral analysis with PCA was successful in differentiating the coffee leaves based on their respective locations that had different climate conditions (Sanchez et al. 2018). Another study had successfully used FTIR spectra analyzed with multivariate statistic method, PCA, to differentiate *Fagus sylvatica* L. from different habitats (Rana et al. 2008). Furthermore, a report showed that FTIR spectroscopy in combination with chemometric method was suitable to differentiate the geographical origins of *Gentiana rigescens* (Zhao et al. 2015). Herein, the PCA was used to classify the FTIR spectrum of patchouli oil and crystal samples with varying concentrations, Figure 5 shows the PCA data plot FTIR of patchouli oil and crystal.

Based on the PCA plot scores in Figure 5, it can be seen that the patchouli oil and crystal samples were classified properly, in which the PCA technique was able to distinguish the FTIR spectrum of patchouli oil with different concentrations. The total variance that can be explained by the PCA plot score is 97% (PC1= 86% + PC2= 11%), and this is such a satisfactorily high variance result. To find out the variables which affect the formation of the PCA plot score, it is shown in the following correlation loading.

Figure 6 shows the variables in the FTIR spectral data of patchouli oil and crystals which have major influence on the formation of the PCA plot score. Based on the information shown by the loading factor, it is visible that PC-1 plot formation on the PCA plot score, in which the patchouli sample on the negative side is PA-100% patchouli crystal. This shows that the formation of patchouli crystal group classification with PA-100% is influenced by the absorption at a wavelength of 2700-2800 cm^{-1}, the relationship between PCA plot scores and further loading is shown in Figure 7.

The biplot analysis shown in Figure 7 shows the main characteristics of the formation of the PCA plot score. Therefore, the characteristics of the PCA plot score can be determined. Based on these results, it can be concluded that the PCA technique using FTIR data is able to distinguish patchouli oil and crystal with different concentrations. However, it is necessary to look for other data preprocessing methods to produce better PCA plots. The effectiveness of FTIR-PCA analysis has been reported by several previous researchers, in which the combination of FTIR PCA has been successfully used to distinguish essential oil samples from plants of the *Lamiaceae* and from the *Asteraceae* (Kustrin et al. 2020). In addition, this method has also succeeded in examining patchouli oil counterfeiting (Fahmi & Mudasir 2020).

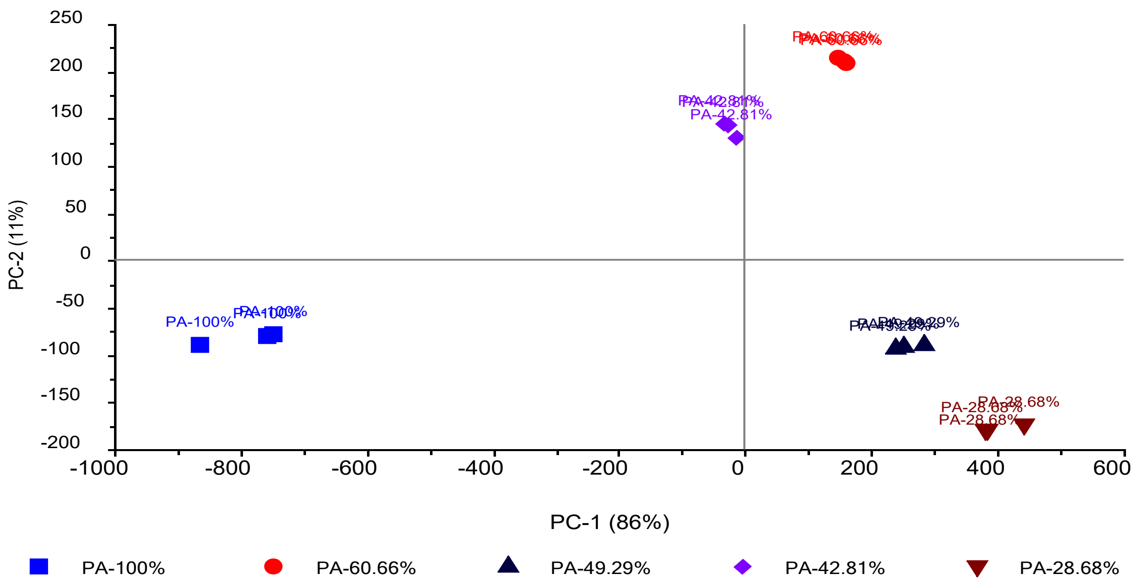

FIGURE 5. PCA plot score of patchouli oil and crystal at different concentrations

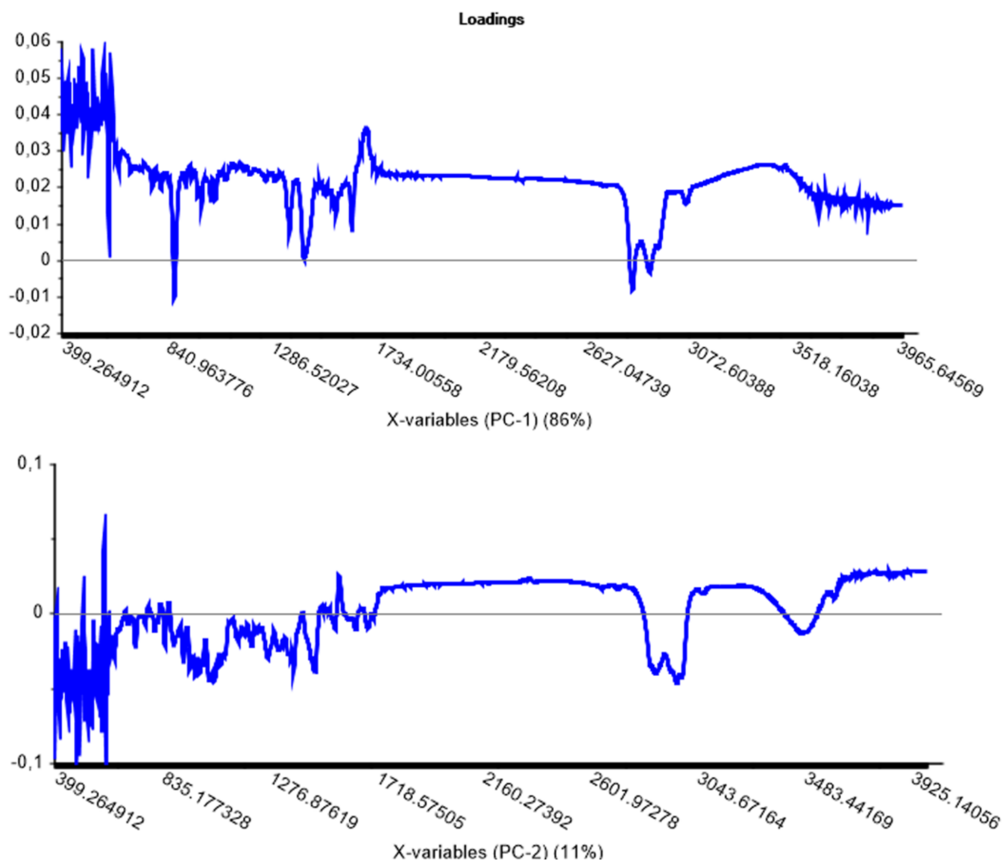

FIGURE 6. Loading factors which configurate the plot scores of PC-1 and PC-1 patchouli oil

LINEAR DISCRIMINANT ANALYSIS (LDA)

The difference between PCA and LDA is that PCA is used to reduce the dimensions of a variable by constructing a principal component (PC), while LDA is used to maximize the separation between classes by finding a linear discriminant function, which is a linear combination of a set of variables (Hassan et al. 2021). LDA is a supervised classification technique, in which the category of this study is predefined patchouli oil (Arslan 2018). The results of the LDA analysis in this study are shown in Figure 8.

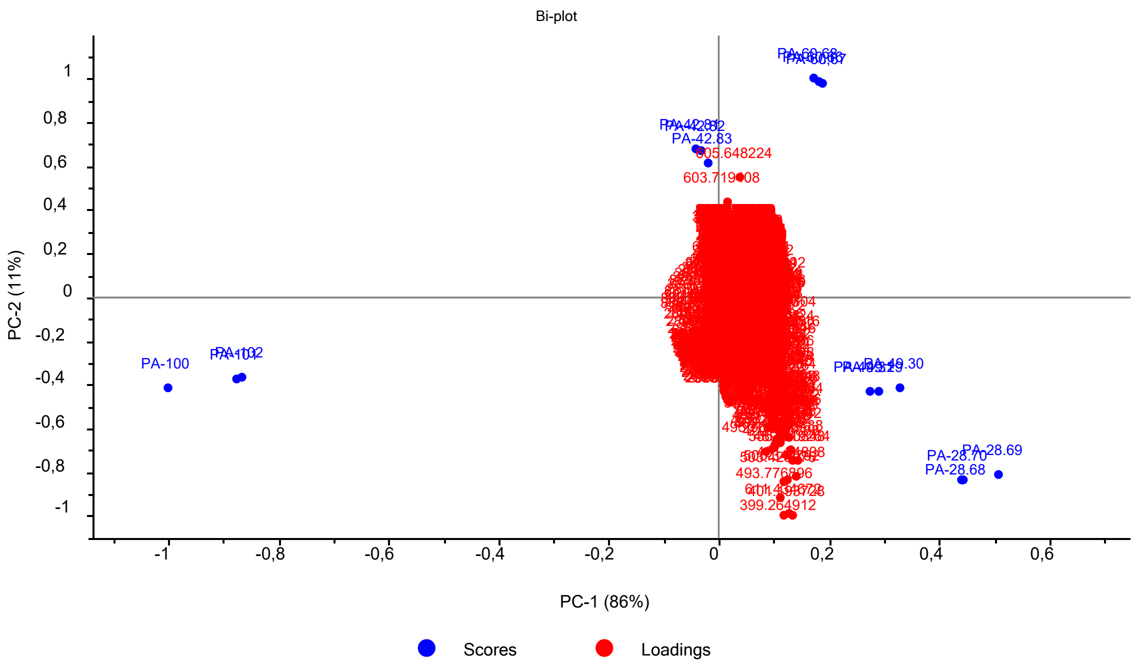

FIGURE 7. Biplot (PCA plot score + *Loading factor*) of the FTIR spectrum of patchouli oil and crystal illustrating the PCA plot score formation factor

To test the LDA model, cross-validation was conducted. The training data set and the test data set were the same data whose identity was already known. Based on the results of cross-validation, all data on the spectrum of patchouli oil and crystal were successfully classified accurately. Each sample formed a group according to the content of PA concentration. Figure 9 shows the confusion matrix which shows the results of the cross-validation which has been done.

Based on the analysis results obtained, it can be concluded that the FTIR-Chemometric technique can distinguish patchouli oil/crystal with different concentrations adequately. Of the three chemometric techniques used, all techniques showed good and accurate results. The results of this study are in line with research conducted by Rafi et al. (2018) which stated that the results of research conducted using the LDA method succeeded in distinguishing several plant species from the same family. Another study stated that the LDA analysis was successful in classifying patchouli oil and those contaminated with castor oil, and those contaminated with palm oil, with an accuracy rate of 100%. Misclassification can occur if the variables used are not appropriate or the oil studied (real oil and fake oil) has a very close resemblance (Fahmi & Mudasir 2020). This indicates that LDA is an effective tool for oil classification for authentication purposes.

CONCLUSION

This research was conducted to differentiate the concentration of patchouli oil by using the FTIR-Chemometric combination method (HCA, PCA and

LDA). The results of the HCA and PCA analysis were successfully used to observe the pattern of FTIR spectrum classification of patchouli oil, while the results of the LDA analysis were successful in showing the discriminated results between concentrations. The results of cross-validation carried out on all patchouli oil/

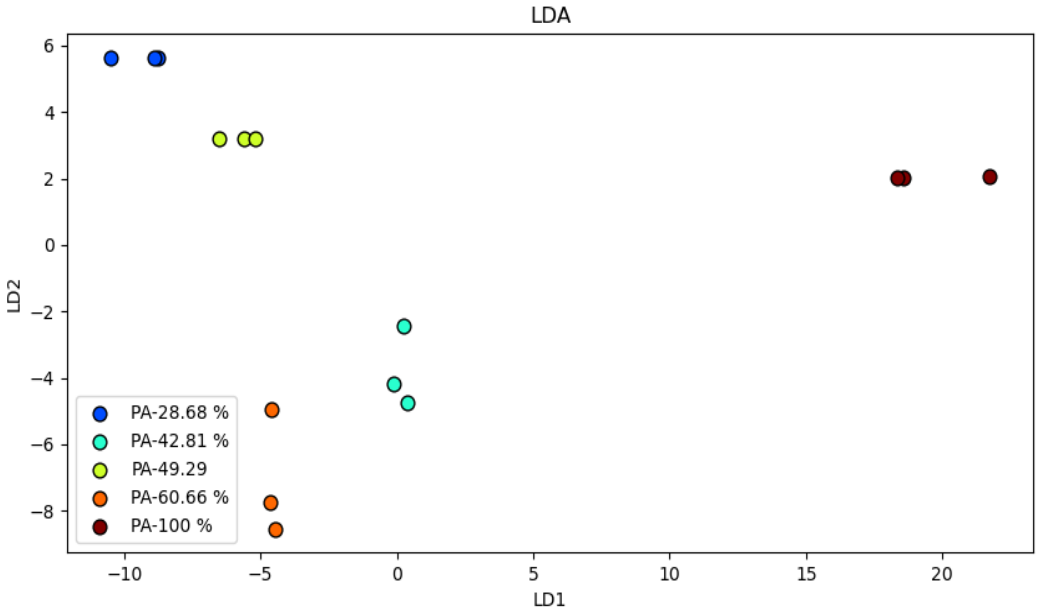

FIGURE 8. LDA plot of the FTIR spectrum of patchouli oil and crystal with different concentrations of patchouli alcohol

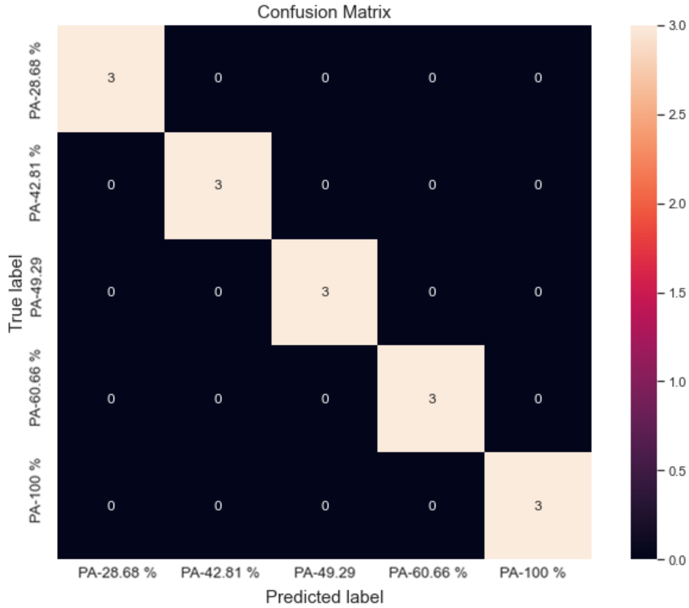

FIGURE 9. Results of cross-validation of classification with LDA method

crystal samples can predict the concentration accurately. Based on the results of this analysis, it can be concluded that the FTIR-Chemometric combination is a fast, easy, accurate and inexpensive method. Moreover, this analysis can be carried out without damaging the sample.

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