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Authentication of Lemongrass (*Cymbopogon citratus*) and Citronella (*Cimbopogon nardus)* Essential Oils in Fragrance Products using FTIR Spectroscopy Combined with Chemometrics

Nina Salamah[1,2]*, Karunia Nining Handaningrum[1], Any Guntarti[1], ABM Helal Uddin[3] and Mustofa Ahda[1,2]
[1]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[2]Ahmad Dahlan Halal Center, Universitas Ahmad Dahlan, Yogyakarta 55164, Indonesia
[3]The Kulliyyah of Pharmacy, International Islamic University Malaysia, Malaysia
*Corresponding author (e-mail: nina.salamah@pharm.uad.ac.id)

Lemongrass plants are natural herbs that contain essential oils, which have been used as aromatic ingredients in the pharmaceutical, perfume, and culinary industries. As lemongrass oil is expensive to manufacture, they are frequently adulterated with other oils. Therefore, commercial oil products should undergo authentication analysis. The purpose of this study is to authenticate lemongrass essential oil in commercial products using Fourier Transform Infrared Spectroscopy (FTIR) and chemometrics. Lemongrass oil was extracted through steam and water distillation methods. These were used as raw essential oils in the reference product formulations. These formulations were analysed by FTIR spectroscopy, and the spectra obtained were processed quantitatively using the PLS method and qualitatively using the PCA method. The PLS model was the most optimal at 3383-2270 cm^{-1}, with R^2 values of 0.9985, 0.9999, and 0.9989, and an RMSEC value of 1.25 %. Discriminant analysis found that the commercial product contained citronella and lemongrass oils.

Keywords: Authentication; lemongrass; essential oil; FTIR; Chemometrics

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Essential oils are recognized in the agribusiness sector for producing high-value domestic and export products. Indonesia, currently a major player in the international spice trade, is one of the world's largest producers of essential oils. These oils are found in many plants, including citronella, vetiver, patchouli, nutmeg, cinnamon, eucalyptus, sandalwood, and clove [1, 2]. Essential oils are derived from various parts of a plant, including leaves, flowers, fruits, seeds, stems, bark, and roots. Also known as etheric oil, an essential oil is a type of vegetable oil that is volatile at room temperature but does not decompose. It is a thick liquid with a unique aroma, and is easily soluble in organic solvents but insoluble in water [2]. Essential oils consist of hydrocarbon groups, including terpenes, aromatics, olefins, paraffins, and oxygenated hydrocarbon compounds. Lemongrass is a plant that produces essential oil. It is commonly used in Indonesia for daily purposes, as a spice etc. [3].

The use of lemongrass oil is rapidly increasing. It is highly likely to be adulterated with other oils. This is because it has lower production yields compared to other essential oils [4]. Pratiwi et al. [5] stated that the lemongrass oil in several products in the market was identified as adulterated, especially those that showed profiles similar to turpentine oil. Bota et al. [6] stated that citronella oil products sold in the market were no longer 100 % pure, due to the addition of compounds such as α-pinene, δ-carene, and β-pinene, which are the main compounds of turpentine oil.

Authentication is critical in protecting the industry and consumers from fraud and counterfeiting, as these products may harm consumers. Analysis of counterfeits is often constrained by the complexity of ingredient mixtures and the similar chemical properties of certain ingredients, which can affect the authenticity of the product [7]. Product authorship can be performed by various methods, including polymerase chain reaction (PCR), enzyme-linked immunosorbent assay, differential scanning calorimetry (DSC), mass spectrometry, chromatography, and spectroscopy. These methods can accurately isolate and quantify components with similar chemical structures, but they often require much time and complex sample preparation. So, a method that can detect adulteration, quickly, and accurately is needed, namely, Fourier transform infrared (FTIR) spectroscopy [8].

This study employed FTIR spectroscopy to identify and analyse the adulterants. FTIR is widely used because it is a simple and quick method for determining the quality of natural materials and categorizing functional groups [9]. Unfortunately, the fingerprint region of the FTIR spectra obtained had a complex pattern, requiring the employment of chemometric methods [10]. This study also identified and categorized lemongrass oil and fragrant essential oils in the market.

EXPERIMENTAL

Chemicals and Materials

The apparatus used was beakers, a set of distillation equipment, a set of Soxhlet extraction tools, and an FT-IR instrument (Thermo Scientific Nicolet iS10). The materials used in this study were kitchen lemongrass and fragrant oils obtained from the traditional market in Yogyakarta city, turpentine oil (Sigma), essential oil products (brands A, B, and C) obtained from e-commerce, and n-hexane (Merck).

Isolation of Distilled Lemongrass Essential Oil

Fresh kitchen lemongrass and fragrant plants were sorted and washed, then dried using an oven at 60 °C for 24 hours. The dried kitchen lemongrass and fragrant plants were put into a distiller for the distillation process. The evaporated oil was collected, and anhydrous Na_2SO_4 was added to reduce the water content of the oil [5, 11].

Isolation of Essential Oil by Soxhlet Extraction

The stems of the kitchen lemongrass and fragrant lemongrass samples were sorted and washed, then chopped into small pieces. The chopped pieces were then blended. Then, up to 100 g of the blended sample was weighed, wrapped in filter paper, and put into the Soxhlet extraction tool. 250 mL of n-hexane was added in two parts: 150 mL in the Soxhlet flask (round-bottom flask), and 100 mL in the Soxhlet tube to wet the sample. Then, three boiling stones were added, and the tube was mounted on the Soxhlet distillation device. The extraction process was carried out by turning on the heater, which was set to a temperature range of 78-80 °C [12].

FTIR Analysis

Reference samples of the kitchen lemongrass and fragrant essential oils, and turpentine oil, were prepared at the following concentration ratios: 0:100; 20:80; 40:60; 60:40; 80:20; 100:0. Readings were taken with the following parameters: 4000-650 cm^{-1}, resolution: 8, and number of scans: 32. Each analysis was performed in duplicate [13].

FTIR Data Analysis of PLS and PCA Chemometrics Combination

FTIR data were analyzed using multivariate calibration with MINITAB 20 software, employing PCA and PLS techniques for quantification. The processed data used were absorbance spectra and preliminary processed spectra involving all absorption features or some absorption data [14].

RESULTS AND DISCUSSION

Isolation of Lemongrass and Citronella Essential Oils

The drying of the lemongrass sample before extraction aims to reduce the water content so that the distillation and extraction process produces relatively more oil [15]. Drying using an oven can reduce moisture content faster, and the temperature and humidity can be adjusted accordingly [16]. Water-vapour distillation is a process for separating components of a mixture through steam and low pressure. Water vapour and oil will condense and be accommodated in a separating container based on specific gravity [17].

FTIR Spectra of Lemongrass and Citronella Essential Oils

FTIR is a method used to detect functional groups, identify organic compounds both qualitatively and quantitatively, and analyse mixtures in samples without damaging them [18]. Figure 1 shows the FTIR spectral profiles of turpentine, kitchen lemongrass, and fragrant oils measured in triplicate.

Figure 1 shows the FTIR-ATR spectral profiles of kitchen and fragrant oils, which were obtained by two different extraction processes, namely distillation and Soxhlet extraction. A typical spectral pattern can be seen, as all the samples had similar spectra, with the only difference being the absorbance values. The functional groups detected in the spectra are listed in Table 1. Based on this, the functional groups in turpentine oil were clearly different from the other samples.

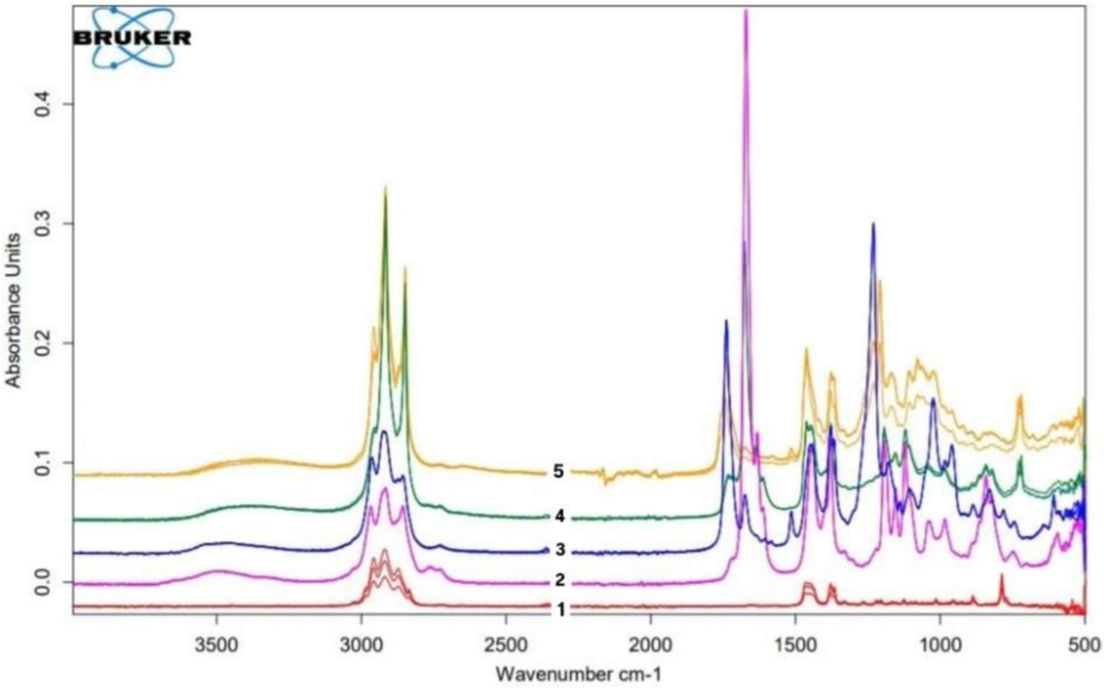


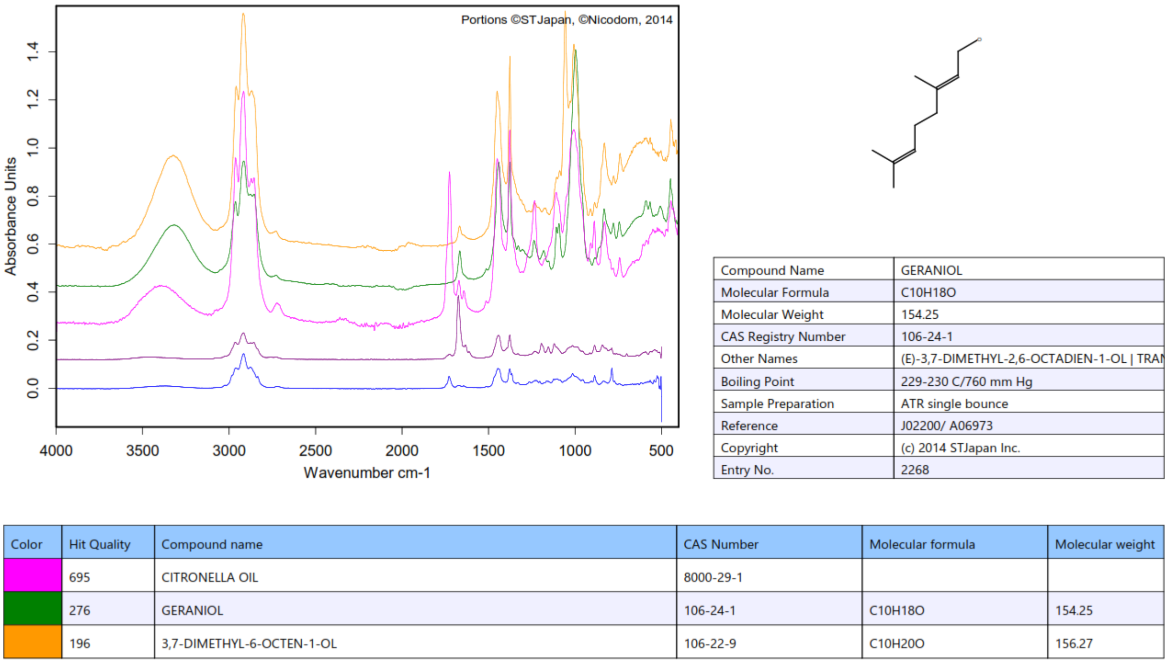
Figure 1. FTIR spectra of turpentine (1), lemongrass and citronella by distillation (2-3); lemongrass and citronella by Soxhlet extraction (4-5).

Table 1. Functional groups in the spectra of lemongrass and citronella oils.

Wavenumbers (cm^{-1})					Wavenumbers (cm^{-1}) literature [19]	Shape/Intensity	Functional Group
Distilled lemongrass oil	Distilled citronella oil	Lemongrass oil Soxhlet result	Citronella oil Soxlet result	Turpentine Oil			
3400	3400	3400	3400	-	2400-3600	Widened/weak	OH
1642	-	1650	-	-	1640-1680	Sharp/medium	C=C
2952	2952	2951	2951	2955	2850-2975	Sharp/Medium	C-H aliphatic
-	1690	-	1700	-	1660-1820	Sharp / Strong	C=O carbonyl
2852 and 2750	2852 and 2750	2852 and 2750	2852 and 2750		2850 and 2750	Sharp/medium	aldehyde

Based on Table 1, the functional groups possessed by the samples are similar to the functional groups possessed by citronella compounds, with a hit quality value greater than citronellol and geraniol. This was confirmed from the FTIR spectra of citronella and kitchen lemongrass oil samples, which were compared with the FTIR spectra of citronella compounds in the FTIR-ATR instrument library. Based on Figure 2, comparison of the FTIR spectra of the kitchen and fragrant lemongrass oil samples with the citronella FTIR spectra gave a hit quality number of 695, which implies that the kitchen and fragrant lemongrass oil samples were dominated by these compounds. This is in line with previous research [20]. The largest component of citronella oil is citronella.

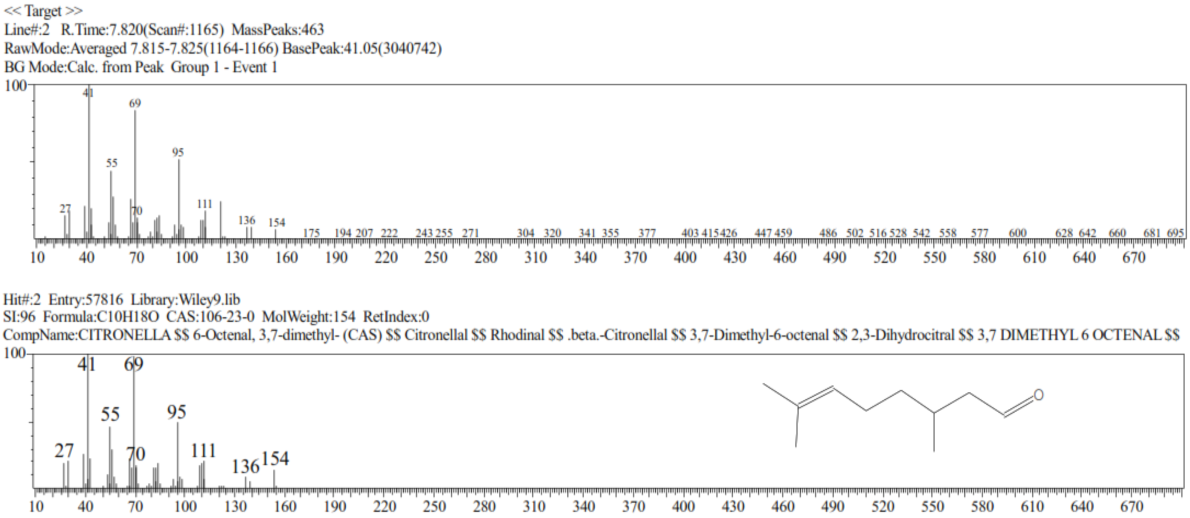
This is also in accordance with the results from Gas Chromatography-Mass Spectroscopy (GC-MS) analysis, which showed the presence of citronella compounds in lemongrass essential oil with a similarity index (SI) value of 96 %, as illustrated in Figure 3.



Compound Name	GERANIOL
Molecular Formula	C10H18O
Molecular Weight	154.25
CAS Registry Number	106-24-1
Other Names	(E)-3,7-DIMETHYL-2,6-OCTADIEN-1-OL | TRA
Boiling Point	229-230 C/760 mm Hg
Sample Preparation	ATR single bounce
Reference	J02200/ A06973
Copyright	(c) 2014 STJapan Inc.
Entry No.	2268

Color	Hit Quality	Compound name	CAS Number	Molecular formula	Molecular weight
🟪	695	CITRONELLA OIL	8000-29-1		
🟩	276	GERANIOL	106-24-1	C10H18O	154.25
🟧	196	3,7-DIMETHYL-6-OCTEN-1-OL	106-22-9	C10H20O	156.27

Figure 2. FTIR spectra of citronella oil (blue) and lemongrass (red), compared to the spectra of citronellol (orange), geraniol (green), and citronella (purple).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<< Target >>
Line#:2   R.Time:7.820(Scan#:1165)  MassPeaks:463
RawMode:Averaged 7.815-7.825(1164-1166) BasePeak:41.05(3040742)
BG Mode:Calc. from Peak  Group 1 - Event 1
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Hit#:2  Entry:57816  Library:Wiley9.lib
SI:96  Formula:C10H18O  CAS:106-23-0  MolWeight:154  RetIndex:0
CompName:CITRONELLA $$ 6-Octenal, 3,7-dimethyl- (CAS) $$ Citronellal $$ Rhodinal $$ .beta.-Citronellal $$ 3,7-Dimethyl-6-octenal $$ 2,3-Dihydrocitral $$ 3,7 DIMETHYL 6 OCTENAL $$
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Figure 3. Mass spectra obtained from GC-MS testing based on Wiley9.lib consisted of Citronella (SI 96 %).

Figures 4 and 5 show the differences in the spectra of the kitchen lemongrass and fragrant lemongrass samples obtained from distillation and Soxhlet extraction with commercially available lemongrass and fragrant oils. The spectrum of distilled kitchen lemongrass oil showed that the sample had a distinctive spectral pattern, which generally reflected the content of pure volatile compounds such as citral, an aldehyde compound with a carbonyl group (C=O) that appears around 1700 cm^{-1}. The spectra of the market lemongrass oil and those of the brands BS, TS, and OT showed differences in the intensity and position of certain peaks. These differences may indicate variations in composition and the addition of other ingredients in the market samples. Distilled citronella oil showed characteristics of pure essential oils, with significant peaks at around 3400 cm^{-1} indicating the presence of hydroxyl groups (O-H) from citronellol compounds, and the sharp peak at 1700 cm^{-1} indicating the presence of carbonyl groups (C=O) in citronellal, the main component of citronella oil. In addition, there were

C-H vibrations in the range of 2800-3000 cm^{-1}, indicating the presence of aliphatic compounds [21]. In contrast, the spectrum of the market product showed some significant differences. The spectra of lemongrass oil from Soxhlet extraction showed differences in the main peaks, which are usually caused by the solubilization of non-volatile compounds during the solvent extraction process.

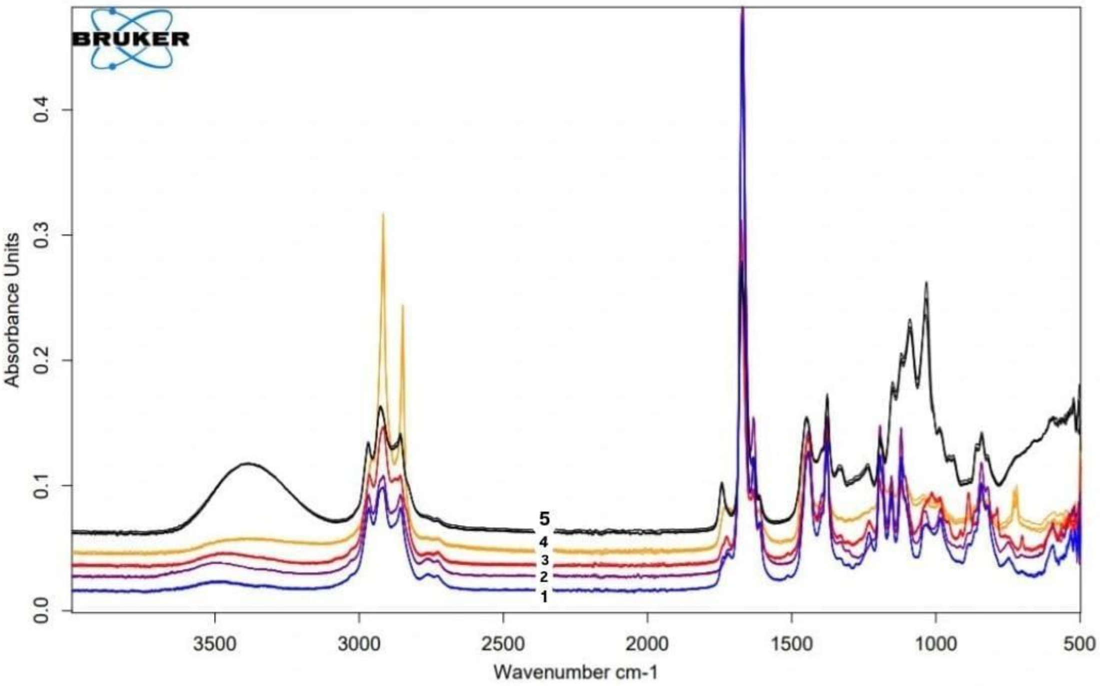

Figure 4. FTIR spectra of distilled lemongrass (1), Soxhlet extracted lemongrass (4) and market samples A (2), B (3) and C (5).

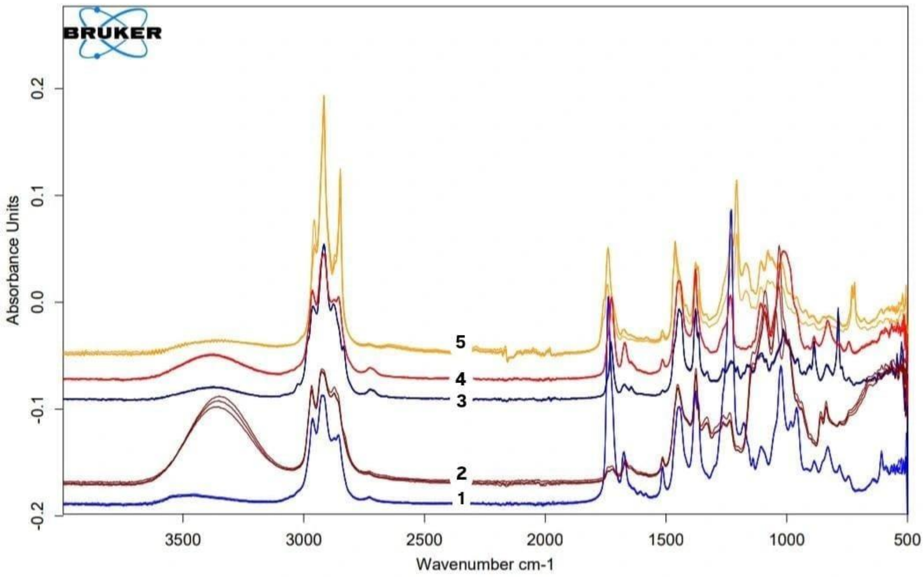

Figure 5. FTIR spectra of distilled citronella (1), Soxhlet extracted citronella (5) and samples of fragrant lemongrass brands A (2), B (3) and C (4).

Analysis of infrared spectral data was performed using multivariate statistical methods. This method, known as the chemometric method [22], is able to extract important information from the infrared spectrum that can be used for qualitative and quantitative analysis purposes. Principal component analysis (PLS) is a method that combines several characteristics that exist in the main components with multiple linear regression. The PLS method aims to analyse and interpret the relationships between independent variables. In practice, the PLS method gives more accurate results because it produces new variables derived from linear combinations between independent variables with smaller dimensions (23). Before conducting PLS analysis, optimization is carried out by determining the wavenumbers that will be used for calibration and validation. The determination of wavenumbers provides a good correlation between the actual value of the tested oils against the predicted value using FTIR, which is expressed by an R^2 (coefficient of determination) value close to 1. Optimization of wavenumbers for the essential oil samples can be seen in Table 2.

Table 2. Wavenumber optimization for PLS calibration of lemongrass and citronella showing the relationship between the x-axis (actual values) and the y-axis (predicted values).

Wavenumber (cm^{-1})	Coefficient of determination (R^2)	Regression Equation	RMSEC
900-800	0.9964	y = 0.9967x + 0.1814	1.87
1300-1100	0.9921	y = 0.9921x + 0.4318	2.81
1400-1320	0.9973	y = 0.9973x + 0.1499	1.70
2900-2800	0.9959	y = 0.9959x + 0.2245	2.08
3383-2270	**0.9985**	**y = 0.9985x + 0.0806**	**1.25**

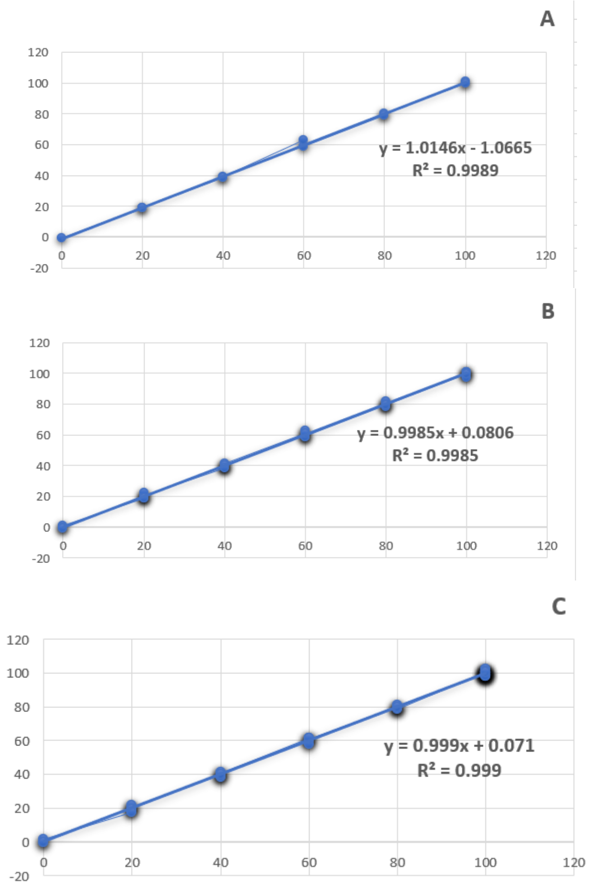

Figure 6. Correlation curve between x-axis (actual values) and y-axis (predicted values) of Lemongrass Oil and Citronella: (A) calibration model using the 3380-2270 cm^{-1} region, (B) internal validation, (C) external validation.

Table 2 shows that the R^2 value for 3383-2270 cm^{-1} was 0.9985, which gave the best predicted and actual values in this region. In addition, the RMSEC (Root Mean Squared Error of Calibration) had a higher accuracy due to its low value. With the high R^2 value and low RMSEC, the authentication of kitchen and fragrant lemongrass oil is considered accurate and precise [24].

Next, the prediction model was evaluated using the cross-validation method by applying the leave-one-out technique. With this technique, one data point is deleted, and a new model will be created using the remaining data. This method is known as internal validation with parameters in the form of RMSECV (Root Mean Square Error of Cross Validation) values. The results are good if the RMSECV produces a low value, and the coefficient of determination (R^2) is close to 1 [22]. The relationship curve between the x-axis (actual value) and y-axis (predicted value) using internal validation is shown in Figure 6.

Based on Figure 6, the R^2 value is 0.9989, and the RMSECV value is 1.25 %, with the regression equation obtained being y = 0.9985x + 0.0806. The small RMSECV value indicates that the model produced low errors and good predictions. External validation was then conducted to determine whether the prediction model could be applied to new samples based on R^2 and RMSEP (Root Mean Square Error of Prediction) values. Figure 4 shows that external validation produced a regression equation y = 0.9985x + 0.0806 with R^2 = 0.9989 and RMSEP = 0.889. The R^2 value close to 1 and the low RMSEP value indicate the model was accurate and reliable for creating new samples. From the calibration results, internal validation and external validation proved to have good and acceptable results, because they had high predicted and actual values (R^2 close to 1) and a good level of accuracy and precision.

The FTIR spectra were then analysed using the Principal Component Analysis (PCA) method. PCA is a statistical technique that is applied to one variable when finding variables that are related. The main objective is to explain as much variation as possible in the original data by using the fewest possible principal components, referred to as factors [25]. The results of the analysis using PCA at 3383-2270 cm^{-1} reduced the initial data, which had 100 PC variables, to three new variables (PC1, PC2, PC3, and PC4), which could provide 100 % data information. PC can explain variations in data, thus facilitating data analysis and visualization (Salamah et al., 2024). The PCA score plot is a latent variable. Samples that have the same or similar values in the main components have similar physicochemical properties [26].

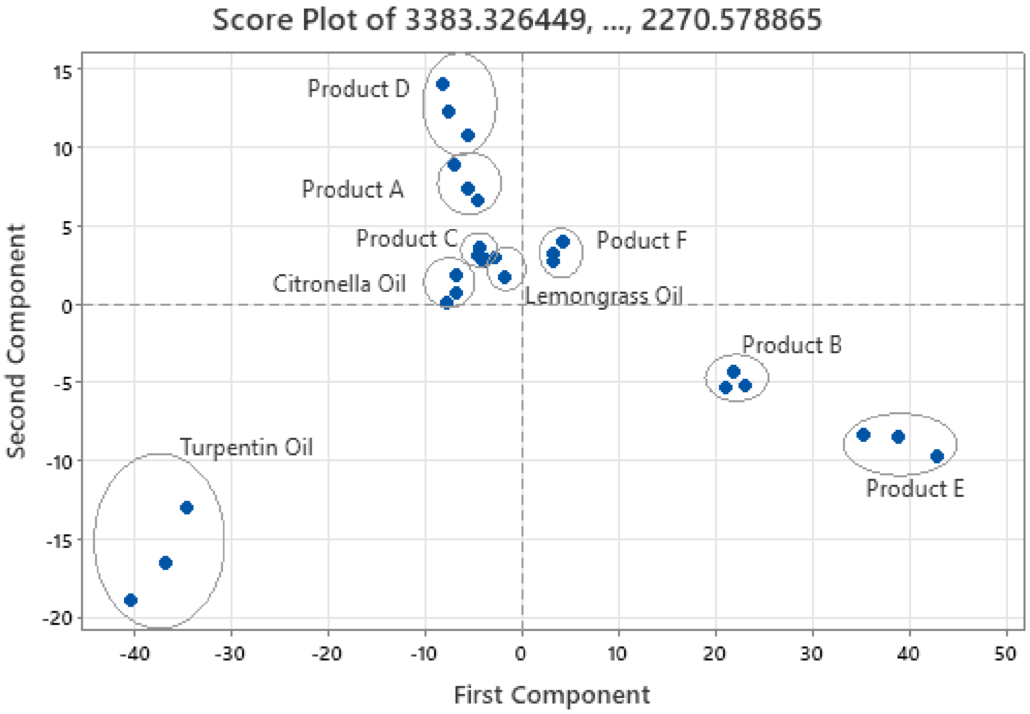

Figure 7. PCA Score Plot of lemongrass essential oil, citronella oil, turpentine oil, and market products A, B, C, D, E, and F.

The PCA score plot in Figure 7 shows that the kitchen lemongrass essential oil sample was in the same quadrant as citronella oil, as well as products A, C, and D, which indicates they all had similar physicochemical characteristics. On the contrary, products B and E were in different quadrants; thus, these two products were likely not pure citronella oil. Still, there was a mixture of other oils, which did not contain turpentine oil. Adjacent points on the score plot indicate that the samples had similar physicochemical properties based on the principal components. This analysis shows that PCA was able to distinguish between essential lemongrass oils from kitchen lemongrass, fragrant lemongrass, turpentine oil, and market products. PLS and PCA methods were able to effectively differentiate between kitchen and fragrant lemongrass essential oils and determine the compositional similarity with commercial products.

CONCLUSION

The FTIR spectra of citronella and kitchen oils indicated functional groups that had similarities with citronellol and citronellal. This could also be seen from the PCA analysis results, in which both samples were in the same quadrant, suggesting similarities in their chemical and physical properties. Meanwhile, of the six commercial products that claimed to contain lemongrass oil, only products A, C, and D had results in the same quadrant as the citronella and kitchen essential oils. The other three products were found in different quadrants, so they are not likely to be pure lemongrass oil, but also not turpentine oil. FTIR analysis combined with chemometrics gave good results and could effectively distinguish between kitchen and fragrant lemongrass essential oils and determine their similarity with market products.

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