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Judul artikel : Fourier Transform Infrared Spectroscopy combined with chemometrics for quality control of *Curcuma aeruginosa* rhizomes: An essential oil analysis

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Penulis : Bekti Nugraheni, Abdul Rohman, Ratna Asmah Susidarti, dan Purwanto

**Tabel Tahapan Publikasi Artikel**

| No | Tahapan Publikasi                                                                                                                                                                                                                                                                                                             | Tanggal          |
|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| 1  | Submission of manuscript                                                                                                                                                                                                                                                                                                      | 6 Juni 2024      |
| 2  | Follow-up on manuscript revision request                                                                                                                                                                                                                                                                                      | 9 Juni 2024      |
| 3  | APC Payment request from TJNPR editorial                                                                                                                                                                                                                                                                                      | 19 Juni 2024     |
| 4  | APC payment report                                                                                                                                                                                                                                                                                                            | 28 Juni 2024     |
| 5  | Revised Manuscript                                                                                                                                                                                                                                                                                                            | 4 September 2024 |
| 6  | Follow up on Revised Manuscript                                                                                                                                                                                                                                                                                               | 5 September 2024 |
| 7  | Check Galley Proof                                                                                                                                                                                                                                                                                                            | 16 Oktober 2024  |
| 8  | Article Published Vol 15 issue 4 (Oktober 2024) on Website<br><a href="https://www.ovid.com/jnls/japtr/fulltext/10.4103/japtr.japtr_75_24~fourier-transform-infrared-spectroscopy-combined-with">https://www.ovid.com/jnls/japtr/fulltext/10.4103/japtr.japtr_75_24~fourier-transform-infrared-spectroscopy-combined-with</a> | 19 Oktober 2024  |

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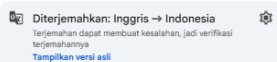
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[Mahesh Sawant](mailto:Mahesh.Sawant@wolterskluwer.com) ([he/him/his](mailto:he/him/his))

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Dear Mr. Mahesh Sawant

Thank you for your email

The exact statement on page number 255 is:

According to Sieniawska et al. (2018), the beta-element in *Nigella damascena* has moderate to strong antibacterial activity against *Mycobacterium tuberculosis* <sup>[31]</sup>. The essential oil of *Neomirandea angularis* leaves contains *Bacillus cereus*, which is the target of germacrene D's antibacterial activity <sup>[12]</sup>.

So, the correct reference on page number 257 is:

31. Sieniawska E, Savicki R, Golus J, Swatko-Ossor M, Gmalika G, Skaliczka-Wozniak K. *Nigella damascena* L. essential oil-a valuable source of  $\beta$ -element for antimicrobial testing. *Molecules*. 2018;23(2).

32. Washington VD, Palazzo MC, Haber WA, Setzer WN. The chemical composition and antibacterial activity of the leaf essential oil of *neomirandea angularis* (asteraceae) from monterverde, Costa Rica. *J Essent Oil-Bearing Plants*. 2010;13(1):108-11.

Thank you very much.

Best regards  
Bekti Nugraheni  
Author

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 **Sawant, Mahesh** <Mahesh.Sawant@wolterskluwer.com>  
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Dear Mr. Mahesh Sawant

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Best regards,  
Bekti Nugraheni

## 8. Article Published Vol 15 issue 4 (Oktober 2024) on Website

< Previous Issue Next Issue >



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Dyan Wigati, Erna Prawita Setyowati, Sylvia Utami Pratiwi, Ari Satia Nugraha

*Journal of Advanced Pharmaceutical Technology & Research*. Oct-Dec 2024; 15(4):243-247

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#### **Fourier Transform Infrared Spectroscopy combined with chemometrics for quality control of *Curcuma aeruginosa* rhizomes: An essential oil analysis**

Bekti Nugraheni, Abdul Rohman, Ratna Asmah Susidarti, Purwanto Purwanto

*Journal of Advanced Pharmaceutical Technology & Research*. Oct-Dec 2024; 15(4):248-257

### BUKTI KORESPONDENSI MANUSCRIPT SUBMISSION

#### **FTIR COMBINED WITH CHEMOMETRICS FOR QUALITY CONTROL OF *Curcuma aeruginosa* RHIZOMES: AN ESSENTIAL OIL ANALYSIS**

**Bekti Nugraheni<sup>1,2</sup>, Abdul Rohman<sup>3\*</sup>, Ratna Asmah Susidarti<sup>3</sup>, Purwanto<sup>4</sup>**

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#### **ABSTRACT**

Rhizome of *Curcuma aeruginosa* is a traditional herb utilized in many nations. The compound content in *C. aeruginosa* rhizomes can vary depending on their geographical origin. The purpose of this study was to identify the active components in *C. aeruginosa* essential oil based on several geographical origins and to establish chemical fingerprints for quality control. Chemical fingerprints of *C. aeruginosa* rhizome essential oils from five different geographic origins have been determined using FTIR. Principal Component Analysis (PCA) and Partial Least Squares Discriminative Analysis (PLS-DA) were utilized to examine the chemical data of *C. aeruginosa* rhizome essential oil utilizing pattern recognition techniques. A total of 52 fluctuating compounds were identified in GC-MS instrumentation, the highest essential oil result was the 1,8-cineole compound content. The *C. aeruginosa* rhizome samples were successfully grouped based on their geographical origins, according to the consistent results from the FTIR analysis. Wavenumber 1489.0715, 1475.3892, 1520.7538, and 1653.0743 cm<sup>-1</sup>

were the characteristic components that can distinguish samples from different geographical origins and for quality control. This work showed that chemometric methods in conjunction with FTIR-based fingerprinting can offer a dependable platform for differentiating plants of various origins, which benefits quality control.

**Keywords:** *C. aeruginosa*, Chemometric, Essential oil, FTIR, GC-MS

## INTRODUCTION

Indonesia, as an agricultural country with a tropical climate, has various types of natural wealth, including natural wealth in the form of plants, one of which is *Curcuma aeruginosa*. *C. aeruginosa* belongs to the *Zingiberaceae* family, the most widely used part of the plant is the rhizome as a traditional medicine in India and Southeast Asia for various diseases, including gastrointestinal disorders as well as immune and respiratory-related disorders<sup>[1,2]</sup>. In Indonesia, *C. aeruginosa* can grow at a height of 5-750 meters above sea level, usually growing wild, especially in teak forests and grasslands. Differences in where medicinal plants grow can cause variations in their chemical contents and biological activities<sup>[3]</sup>. Therefore, it is very important to develop a chemical analysis method that makes it easier to determine the chemical differences of *C. aeruginosa*, especially the active substances of its essential oil.

*C. aeruginosa* is one of the many varieties of natural wealth that Indonesia possesses as a tropical agricultural nation with a varied environment. In India and Southeast Asia, *C. aeruginosa* is used as a traditional medicine for a variety of ailments, including digestive and immune system issues as well as respiratory and digestive-related disorders<sup>[1,2,4]</sup>. The rhizome of the plant is the most commonly used part of the plant. *C. aeruginosa* grows naturally in teak forests and meadows and can reach elevations of 5-750 meters above sea level in Indonesia. Biochemical activity and chemical composition of medicinal plants can differ depending on where they are grown<sup>[3]</sup>. To ascertain the chemical differences of *C. aeruginosa*, particularly the active substances of its essential oil, it is crucial to design a chemical analysis method that is easy to use.

Monoterpenes and sesquiterpenes make up the majority of the essential oil made of *C. aeruginosa* rhizomes found in various places<sup>[5]</sup>. *C. aeruginosa* essential oil has been shown in numerous studies to have health benefits as an antioxidant, antibacterial, anti-inflammatory, and anticancer agent<sup>[6,7]</sup>. It can also be utilized in food packaging, pharmaceutical applications, and functional foods. In order to assess variances in the quality of *C. aeruginosa* rhizomes, it is vital to examine the chemical composition of *C. aeruginosa* essential oils based on geographical origins.

Currently, a number of spectroscopic and chromatographic methods have been widely used for the quality control of herbal medication. Since essential oils are the primary bioactive component of *C. aeruginosa* rhizomes, it is possible to effectively define the properties of the herbs using the chemical components found in the essential oils. The analysis of essential oil constituents is a common use of Fourier Transform Infrared Spectroscopy (FTIR) fingerprint technology<sup>[8,9]</sup>. The fingerprint method uses pattern recognition technology to identify different chemical components in sample fingerprints. It may also be used as a quality criterion for herbs based on similarity values.

Several studies on plant chemical diversity have so far been carried out with the help of chemometric

techniques<sup>[10,11,12]</sup>. Hierarchical cluster analysis (HCA) is a method in statistics and data analysis that is used to categorize objects or data into groups based on their level of similarity. However, models created by HCA will miss some information, especially when used for large amounts of data such as analysis of complex herbal compounds or biological activity information. Therefore, to overcome this, Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) are used to reduce the dimensions of the large data, grouping samples more accurately. In the context of classification, PLS-DA is often more relevant and powerful because it was specifically developed to deal with multicollinearity problems. However, in some situations, PCA can also provide valuable information especially when the main focus is on exploration and dimensionality reduction<sup>[13]</sup>.

In the combination of chemometric data processing, samples from different origins can be classified efficiently and the characteristic factors leading to this classification can be determined. Although there are several publications related to the qualitative and quantitative analysis of essential oils in *C. aeruginosa* rhizomes using GC-MS, there are no studies yet. which involves the classification of essential oils of *C. aeruginosa* rhizomes of different origins with the help of chemometric techniques. In this research, FTIR fingerprinting of *C. aeruginosa* rhizome essential oil was carried out for the first time. In this study, the aim was to use FTIR applications combined with chemometrics to classify the essential oil of *C. aeruginosa* rhizomes and to analyze the essential oil of *C. aeruginosa* rhizomes using Gas Chromatography-Mass Spectra (GC-MS).

## **MATERIALS AND METHODS**

### **1. Material**

The samples used were *C. aeruginosa* rhizomes taken from 5 areas, namely Kaloran, Temanggung Regency; Kalibakung, Tegal Regency; Jumantono, Karanganyar Regency; Bayat, Klaten Regency; and Trucuk, Klaten Regency. In addition, this study also used Na<sub>2</sub>SO<sub>4</sub> (Smartlab), hexane (Smartlab), and distilled water.

### **2. Equipment**

FTIR Cary 630 (Agilent), GC-MS QP2010 SE (Shimadzu), analytical balance (Sartorius), centrifuge (Sartorius).

### **3. Method**

#### **Sampling**

The samples used in this research were the rhizomes of *C. aeruginosa* originating from some areas: Kaloran, Temanggung Regency; Kalibakung, Tegal Regency; Jumantono, Karanganyar Regency; Bayat, Klaten Regency; and Trucuk, Klaten Regency, which were harvested in October 2022.

#### **Distillation of essential oils**

A total of  $\pm 8$  kg of wet *C. aeruginosa* rhizomes which had been cut to a thickness of 1 mm was distilled with water steam for 4 hours. Oil distillate obtained was added with *anhydrous Na<sub>2</sub>SO<sub>4</sub>* to remove remaining water traces, then centrifuged at a speed of 4000 rpm for 5 minutes. The clear oil obtained was then stored in an air- and light-tight bottle, and stored in a freezer<sup>14</sup>.

#### **Analysis of essential oil profiles using GC-MS**

The GC-MS tool is used to analyze the essential oils obtained to determine the composition and comparison of

the chemical group compounds that make up the essential oils. The data obtained are chromatograms from GC analysis and mass spectra from MS analysis. The chromatogram shows *database-based* peaks for various compounds in the sample with their respective retention times, peak area percentages, and putative compound names. Mass spectrum shows a peak at a certain retention time and estimates the compound structure and molecular formula with the percentage of the similarity index. The essential oil profiles were analyzed at the Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta using Gas Chromatography-Mass Spectrometer (GC-MS)-QP2010 SE (Shimadzu, Japan). The analysis system conditions included the use of a 30-meter Agilent® capillary column with an inner diameter of 0.25 mm. The carrier gas was helium set at a pressure of 45.9 kPa with a flow rate into the column of 0.80 mL/minute. The injection system used a split injection model with an injector temperature of 175°C and a split ratio of 1:50. The column temperature was programmed (temperature programming) with an initial temperature of 75°C for 3 minutes (hold time) then increased regularly at a rate of 5°C/minute until it reached a temperature of 300°C and held at that temperature for 40 minutes. The detector used in this analysis was a 70 eV EI (Electron Impact) with an ion source temperature of 200°C which is capable of covering m/z 10.00 to m/z 500.00. The oil components were identified by comparing the mass spectrum obtained (target) to data from the software library (library hit) with the highest similarity index. The percentage composition of the oil was calculated based on the peak area in the resulting chromatogram to the total area of the chromatogram.

### **FTIR Spectroscopic Analysis**

The use of the FTIR Spectrophotometer follows<sup>[15,16]</sup>. Spectrum of oil samples was obtained from a Cary 630 Agilent FTIR spectrophotometer (USA) equipped with ZnSe crystals and beam splitter Germanium on KBr substrate, Deuterated Triglycine Sulfate (DTGS) with technique handling of Attenuated Total Reflection (ATR) samples. The spectrometer instrument was integrated with the software *MicroLab Expert* which was run on an HP PC with Windows 10 operating system for spectrum data extraction and analysis. Drops of oil were placed on the ATR crystal, scans were carried out 32 times with a resolution of 8 cm<sup>-1</sup> in wave number ranging from 4000-600 cm<sup>-1</sup>. Each sample was scanned in a total of 5 replications. The signal strength of the instrument was optimized to fall in the range of 25-95%. Basic spectrum recording from air was carried out for each and every sample measurement to avoid spectrum variations over time due to the influence of the surrounding air. The instrument was maintained at temperature and humidity for minimizing water vapor interference. Before the initial scan and every change in sample, ATR crystals were cleaned with ethanol pro analysis 3 times then dried with a soft tissue. The degree of crystal cleanliness was verified by collecting the background spectrum and comparing it with the previous spectrum.

### **Pattern Recognition Analysis**

#### **1. PCA**

PCA is widely used to reduce the dimensions of data by explaining the correlation between a large number of variables in a small number of underlying principal component factors without losing much information. After dimensional reduction of the complex variability of chemical information into PC, the low dimensional PCA

score plot is then visually inspected to see sample separation. In addition, it allows pattern recognition analysis of the data by plotting it in a multidimensional space, using new derived variables as dimensions (factor scores). The goal of PCA is to produce components used as predictors or response variables in subsequent analysis. In this study, the FTIR wave number of the essential oil from *C. aeruginosa* rhizome was normalized and then imported into *Metaboanalyst 6.0 Software*.

## 2. PLS-DA

The PLS-DA technique is a regression extension of PCA, utilizing class information to try to maximize separation between groups of observations<sup>[13]</sup>. Existing models can be used to predict unknown classes of objects based on FTIR. In this section, the data were analyzed with PLS-DA to build a linear discrimination model for classification, and thus to confirm the groups found by HCA. This program was processed using *Metaboanalyst 6.0 Software*. Generally, score plots can distinguish different classes of samples; partial loading plots can reveal the influence of variables on class classification. The variables that have the greatest influence on the score plot are the variables that are farthest from the main variable group. In this study, loading PLS-DA plots were used to find chemical markers for discrimination among different sample classes.

## RESULTS AND DISCUSSION

The distillation process was carried out to obtain the essential oils contained in the rhizomes of *C. aeruginosa*. The distillation was carried out using the steam-water distillation method because it can yield a lot of essential oils. The steam-water distillation method uses a high-pressure steam system and raw materials that are not in direct contact with water. The steam flew through a container containing the raw materials (the rhizomes of *C. aeruginosa*). The steam coming out of the container was connected to a condenser, so the condensate containing oil and water was separated according to the specific gravity of the oil. *The distillate obtained was separated from the oil phase from the remaining water phase using the centrifugation method. The centrifugation method was carried out by breaking the fat-protein bonds by rotating (centrifugal force). After centrifugation, the water phase and oil phase were separated due to a difference in specific gravity between the water and oil phases to obtain pure essential oil naturally without requiring heating. Next, centrifugation was carried out again with an addition of anhydrous Na<sub>2</sub>SO<sub>4</sub> to bind the remaining water that was still in the oil phase to obtain pure essential oil.*

*According to the Indonesian Herbal Pharmacopoeia 2nd edition of 2017, the essential oil in C. aeruginosa rhizomes contain no less than 1.89% v/w<sup>[17]</sup>. The C. aeruginosa rhizomes from the five different areas had essential oil levels below 1.89% v/w. This is because different types of soil and height of growing C. aeruginosa plants can affect oil yield. The results of the % essential oil yield of C. aeruginosa rhizome can be seen in Table 1.*

**Table 1. %Yield *C. aeruginosa* rhizome essential oil**

| No | Area                     | Yield (%) |
|----|--------------------------|-----------|
| 1  | Kaloran - Temanggung.    | 0.108     |
| 2  | Kalibakung - Tegal.      | 0.104     |
| 3  | Jumantono - Karanganyar. | 0.105     |
| 4  | Bayat - Klaten.          | 0.079     |
| 5  | Trucuk - Klaten.         | 0.120     |

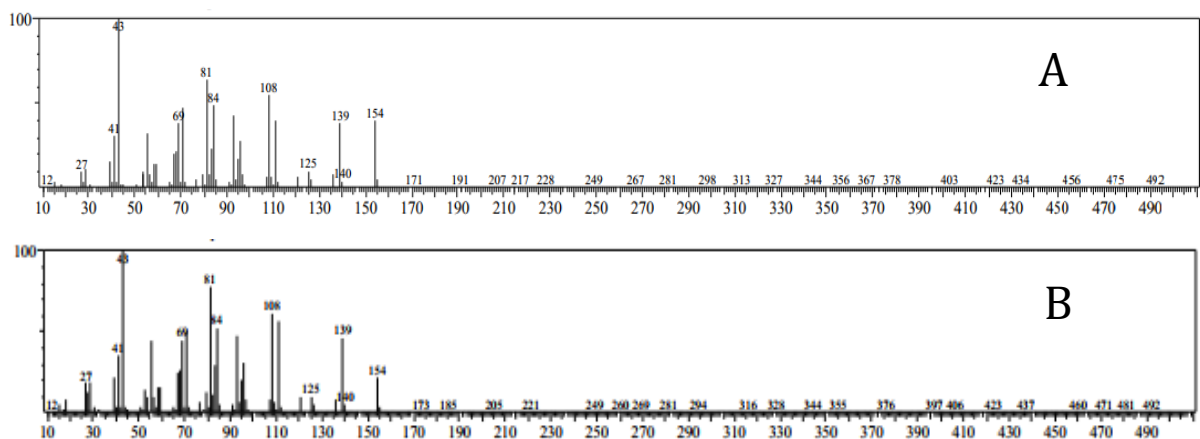
### Essential Oil Content of *C. aeruginosa* Rhizome

The GC-MS method was used to analyze the content of the essential oils in *C. aeruginosa* rhizomes. The essential oils in *C. aeruginosa* rhizomes originating from the five regions contained similar contents but with different levels. Such differences were influenced by the geographical conditions where the plants grew, such as weather, soil height, soil type, soil pH, temperature, and humidity, thus influencing the levels of plant metabolite content<sup>[18]</sup>. The essential oil content of *C. aeruginosa* rhizomes can be seen in Table 2.

**Table 2. Essential oil content of *C. aeruginosa* rhizomes (2 replications)**

| Compound                                                                          | Molecular Weight                               | Concentration (%)           |                           |                                |                       |                        |
|-----------------------------------------------------------------------------------|------------------------------------------------|-----------------------------|---------------------------|--------------------------------|-----------------------|------------------------|
|                                                                                   |                                                | Kaloran, Temanggung Regency | Kalibakung, Tegal Regency | Jumantono, Karanganyar Regency | Bayat, Klaten Regency | Trucuk, Klaten Regency |
| Alpha-Pinene                                                                      | C <sub>10</sub> H <sub>16</sub>                | 0.35                        | 0.24                      | 0.55                           | 0.53                  | 0.65                   |
| Camphene                                                                          | C <sub>10</sub> H <sub>16</sub>                | 0.93                        | 0.73                      | 1.21                           | -                     | 1.58                   |
| Beta-Pinene                                                                       | C <sub>10</sub> H <sub>16</sub>                | 1.28                        | -                         | 1.64                           | 1.52                  | 1.52                   |
| Beta-Myrcene                                                                      | C <sub>10</sub> H <sub>17</sub>                | -                           | -                         | 0.21                           | 0.16                  | 0.19                   |
| 1.8-Cineole                                                                       | C <sub>10</sub> H <sub>18</sub> O              | 26.66                       | 28.38                     | 34.01                          | 29.35                 | 30.93                  |
| Camphor                                                                           | C <sub>10</sub> H <sub>16</sub> O              | 11.47                       | 19.05                     | 3.24                           | 18.37                 | 18.86                  |
| 2-Undecanol                                                                       | C <sub>11</sub> H <sub>24</sub> O              | -                           | 0.47                      | 0.46                           | -                     | -                      |
| endo-Borneol                                                                      | C <sub>10</sub> H <sub>18</sub> O              | 0.70                        | 1.09                      | 0.91                           | 0.92                  | 1.00                   |
| 4-Terpineol                                                                       | C <sub>10</sub> H <sub>18</sub> O              | 0.46                        | 1.00                      | 0.89                           | 0.82                  | 0.90                   |
| Alpha-Terpinyl acetate                                                            | C <sub>12</sub> H <sub>20</sub> O <sub>2</sub> | 0.74                        | 1.75                      | 0.16                           | 1.41                  | 1.50                   |
| Beta-Elements                                                                     | C <sub>15</sub> H <sub>24</sub>                | 0.56                        | 0.49                      | 0.48                           | 0.85                  | 0.58                   |
| Trans-Caryophyllene                                                               | C <sub>15</sub> H <sub>24</sub>                | 0.24                        | 0.18                      | 0.48                           | 0.20                  | 0.18                   |
| Beta-Farnesene                                                                    | C <sub>15</sub> H <sub>24</sub>                | -                           | -                         | 0.28                           | 0.27                  | 0.20                   |
| Alpha-Humulene                                                                    | C <sub>10</sub> H <sub>16</sub>                | 1.83                        | -                         | -                              | 0.21                  | -                      |
| Germacrene D                                                                      | C <sub>15</sub> H <sub>24</sub>                | 0.22                        | 0.18                      | 0.21                           | 0.19                  | 0.26                   |
| Beta-Selinene                                                                     | C <sub>15</sub> H <sub>24</sub>                | -                           | 0.15                      | -                              | 0.14                  | 0.17                   |
| Curzerene                                                                         | C <sub>15</sub> H <sub>20</sub> O              | 0.43                        | 0.41                      | 0.35                           | 0.41                  | 0.41                   |
| Germacrene A                                                                      | C <sub>15</sub> H <sub>24</sub>                | 0.17                        | 0.15                      | 0.16                           | 0.15                  | 0.19                   |
| Germacrene B                                                                      | C <sub>15</sub> H <sub>24</sub>                | 0.17                        | -                         | 0.15                           | -                     | 0.18                   |
| Epiglobulol                                                                       | C <sub>15</sub> H <sub>26</sub> O              | 0.40                        | 0.46                      | 0.40                           | 0.41                  | 0.39                   |
| Elemol                                                                            | C <sub>15</sub> H <sub>26</sub> O              | 0.29                        | 0.26                      | 0.22                           | 0.24                  | 0.30                   |
| Boldenone                                                                         | C <sub>19</sub> H <sub>26</sub> O <sub>2</sub> | 19.06                       | -                         | 14.97                          | 14.53                 | 13.90                  |
| Isocurcumenol                                                                     | C <sub>15</sub> H <sub>22</sub> O <sub>2</sub> | 2.86                        | 8.89                      | 5.97                           | 5.55                  | 4.68                   |
| Juniper camphor                                                                   | C <sub>15</sub> H <sub>26</sub> O              | -                           | -                         | 1.47                           | 1.58                  | 1.94                   |
| 1(10),4-armedenedradiene                                                          | C <sub>15</sub> H <sub>22</sub>                | -                           | -                         | 0.51                           | 0.55                  | 0.54                   |
| Spathulenol                                                                       | C <sub>15</sub> H <sub>24</sub> O              | -                           | -                         | 1.08                           | 1.44                  | 0.15                   |
| Germacrone                                                                        | C <sub>15</sub> H <sub>22</sub> O              | -                           | 3.38                      | 3.14                           | 3.62                  | 3.45                   |
| <i>Aromadendrene epoxide</i>                                                      | C <sub>15</sub> H <sub>24</sub> O              | -                           | -                         | 1.84                           | 0.48                  | 0.50                   |
| Trans-Longipinocarveol                                                            | C <sub>15</sub> H <sub>24</sub> O              | -                           | 0.41                      | 0.57                           | -                     | -                      |
| <i>Germacrol</i>                                                                  | C <sub>15</sub> H <sub>22</sub> O              | -                           | -                         | 3.42                           | -                     | -                      |
| 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-pentyl-3-ol                      | C <sub>15</sub> H <sub>24</sub> O              | 1.44                        | 1.73                      | -                              | -                     | 0.45                   |
| (E)-2.3.3-Trimethyl-2-(3'-methyl-1'.3'-butadiene-1'-yl)-6-methylidencyclohexanone | C <sub>26</sub> H <sub>38</sub> O              | 3.27                        | 2.97                      | -                              | -                     | 2.48                   |
| Tricyclo[5.1.0.0(3.5)]octane-2.6-dione. 1.3.5.7-tetramethyl-                      | C <sub>16</sub> H <sub>28</sub> O <sub>2</sub> | -                           | -                         | 3.69                           | 3.49                  | -                      |
| Cyclohexane. 1.1-dimethyl-2.4-bis(1-methylethenyl)-, cis-                         | C <sub>15</sub> H <sub>26</sub>                | 5.16                        | -                         | 3.84                           | -                     | -                      |
| 2-Cyclohexen-1-one, 4-ethynyl-4-hydroxy-3,5,5-trimethyl-                          | C <sub>10</sub> H <sub>14</sub> O              | -                           | 15.95                     | -                              | -                     | -                      |
| 3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)                             | C <sub>15</sub> H <sub>22</sub> O              | 3.15                        | -                         | -                              | -                     | -                      |

|                                                                                          |                                                |       |       |       |       |       |
|------------------------------------------------------------------------------------------|------------------------------------------------|-------|-------|-------|-------|-------|
| Dihydrocarvone                                                                           | C <sub>10</sub> H <sub>16</sub> O              | -     | 4.01  | -     | 4.47  | 4.33  |
| Calarene                                                                                 | C <sub>15</sub> H <sub>24</sub>                | 5.39  | -     | 3.33  | 3.93  | 4.12  |
| Bicyclo[2.2.2]Octa-2.5-Diene.<br>1.2.3.6-Tetramethyl                                     | C <sub>12</sub> H <sub>18</sub>                | -     | 4.70  | -     | -     | -     |
| Ledenoxide-(II)                                                                          | C <sub>15</sub> H <sub>24</sub> O              | 0.96  | 0.86  | 0.72  | 1.11  | 0.86  |
| Curdione                                                                                 | C <sub>15</sub> H <sub>24</sub> O <sub>2</sub> | 2.76  | -     | -     | -     | -     |
| Ledane                                                                                   | C <sub>15</sub> H <sub>26</sub>                | -     | -     | 2.84  | -     | -     |
| 1H-cyclopropa(a)Naphthalene,<br>1A,2,4,5,6,7,7A,7B-<br>Octahydro-1,1,7,7A-               | C <sub>15</sub> H <sub>24</sub>                | -     | -     | 3.59  | -     | -     |
| (E)-2,3,3-Trimethyl-2-(3'-<br>methyl-1'.3'-butadien-1'-yl)-6-<br>methylidencyclohexanone | C <sub>14</sub> H <sub>22</sub> O              | -     | -     | 2.32  | 2.75  | -     |
| Tricyclo[5.2.2.0(1,6)]undecan-<br>3-ol, 2-methylene-6,8,8-<br>trimethyl-                 | C <sub>15</sub> H <sub>24</sub> O              | 0.66  | -     | -     | -     | -     |
| (3E)-5-Isopropyliden-6-<br>methyl-3,6,9-decatrien-2-one                                  | C <sub>14</sub> H <sub>20</sub> O              | 5.04  | -     | -     | -     | -     |
| trans-p-Mentha-1(7),8-dien-2-<br>ol                                                      | C <sub>10</sub> H <sub>16</sub> O              | 0.89  | -     | -     | -     | -     |
| Hexadecane, 2,6,10,14-<br>tetramethyl-                                                   | C <sub>20</sub> H <sub>42</sub>                | 0.42  | -     | -     | -     | -     |
| Naphthalene, 1,2,3,4-<br>tetrahydro-2,6-dimethyl-7-<br>octyl-                            | C <sub>20</sub> H <sub>32</sub>                | 0.60  | -     | -     | -     | -     |
| Tetradecane, 1-chloro-                                                                   | C <sub>14</sub> H <sub>29</sub> Cl             | 0.90  | -     | -     | -     | -     |
| Phenanthrene. 1.2.3.4.5.6.7.8-<br>Octahydro-                                             | C <sub>15</sub> H <sub>17</sub> NO             | -     | 0.22  | 0.17  | 0.15  | 0.69  |
| Total identified (%)                                                                     |                                                | 99.46 | 98.11 | 99.45 | 99.76 | 98.04 |
| Monoterpene hydrocarbons (%)                                                             |                                                | 4.39  | 0.97  | 3.61  | 2.42  | 3.93  |
| Oxygenated monoterpenes (%)                                                              |                                                | 40.92 | 71.70 | 39.66 | 55.32 | 57.51 |
| Sesquiterpene hydrocarbons (%)                                                           |                                                | 11.91 | 1.15  | 18,19 | 6.28  | 6.41  |
| Oxygenated sesquiterpenes (%)                                                            |                                                | 37.05 | 16.40 | 34.14 | 32.10 | 27.15 |
| Oxygenated diterpenes (%)                                                                |                                                | 1.02  | -     | -     | -     | -     |
| Others (%)                                                                               |                                                | 4.17  | 7.89  | 3.86  | 3.64  | 3.17  |



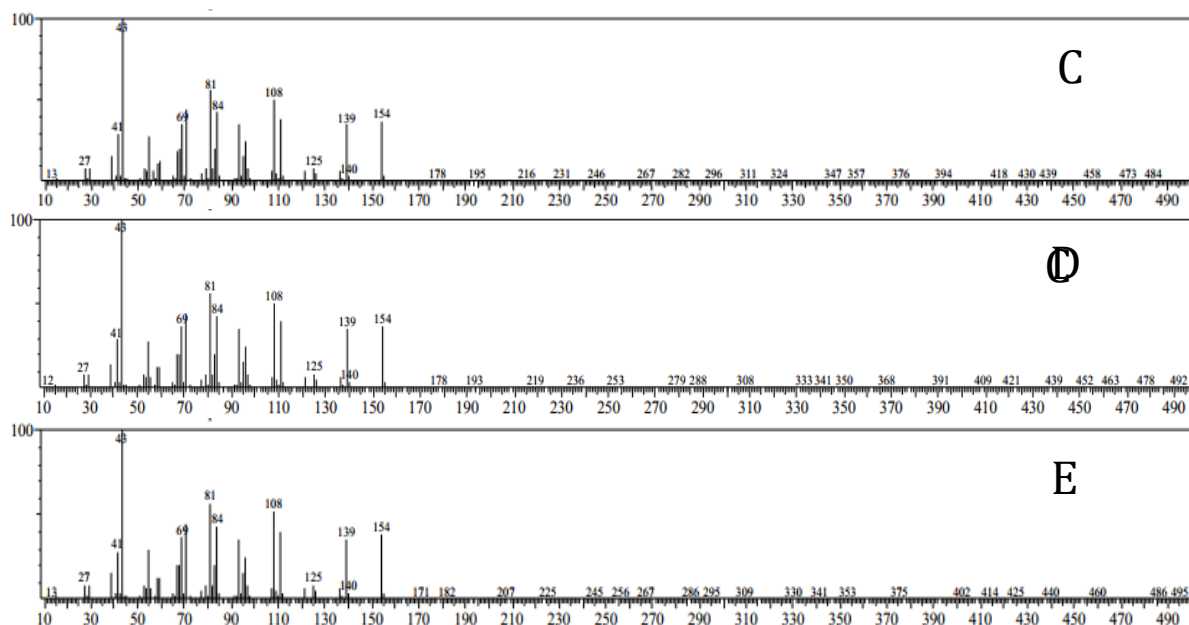


**Figure 1. Representative chromatogram of *C. aeruginosa* rhizome essential oil from different origins: (A). Calories; (B) Kalibakung; (C) Jumantono; (D) Bayat; (E) Trucuk.**

A total of 52 peaks were identified as shown in Table 2, accounting for 98.04–99.76% of the volatile compounds. The main content of the essential oils in *C. aeruginosa* rhizomes from all the regions was almost the same, but there were quite large differences in the composition of the essential oils in the five regions. In Figure 1, the fragmentation patterns of 1,8-cineole compounds from the five regions had different abundances, so the concentrations of the compounds produced were also different. The content of the essential oil in *C. aeruginosa* rhizomes consisted of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. In Table 2, the chemical content of the essential oil in *C. aeruginosa* rhizome was dominated by oxygenated monoterpenes at 40.92–71.70%, followed by oxygenated sesquiterpenes at 16.40–37.05%. The 1,8-cineole compound was found to be the content with the highest level in the essential oils from the five regions, while the camphor and isocurcumenol compounds had different essential oil compositions among the five regions. Variations in the chemical composition of essential oils may be caused by differences in the geographical origins and environmental conditions, which in turn lead to differences in the quality of *C. aeruginosa* rhizome.

### FTIR Spectroscopic Analysis

In the first stage of sample characterization, Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR) is a reasonably quick approach. Easy sample preparation, a broader range of spectrum variations, no requirement for KBr grinding, and insignificant particle size differences are further benefits. The ATR concept, which analyzes the changes in internally reflected infrared photons when they interact with a sample through crystals, diamond, or zinc selenium (ZnSe), is based on the phenomena of total internal reflection. It is not necessary to prepare in advance when using essential oil samples using ATR-IR spectroscopy equipment. Since essential oils constitute a complex mixed system, the findings of the infrared spectrum display the complete overlap of each component's absorption spectrum. For samples of *C. aeruginosa* rhizome essential oil, the typical infrared fingerprint peaks were primarily located between 1750 and 700  $\text{cm}^{-1}$  (Figure 2).

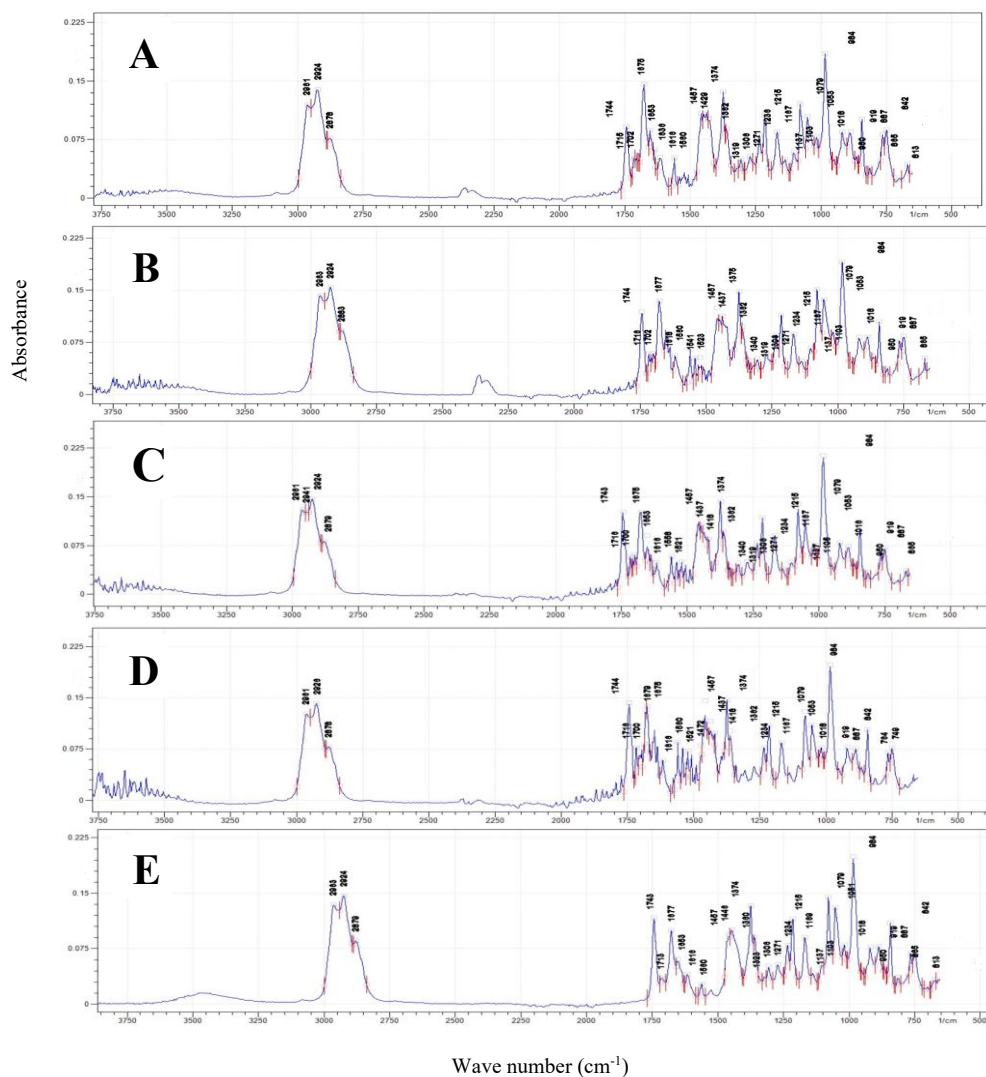


**Figure 2.** *C. aeruginosa* rhizome essential oil from different regions: (A) Caloran; (B) Kalibakung; (C) Jumantono; (D) Bayat; and (E) Trucuk.

Data for each *C. aeruginosa* rhizome essential oil sample was obtained from scanning the results with an FTIR Cary 630 (Agilent) Micro Lab *software* using ATR to measure any changes that were found in the process, reflecting infrared light when the light came to the sample. The advantage of using ATR is that the measurement is non-destructive and does not require complicated sample preparation so the analysis process is faster. Scanning was carried out in 5 replications in waves ranging from 4000-750  $\text{cm}^{-1}$ . Wave number 1750-700  $\text{cm}^{-1}$  was a fingerprint area with a resolution of 4  $\text{cm}^{-1}$ , measurements at a resolution of 4  $\text{cm}^{-1}$  showed that for each measurement distance of 4  $\text{cm}^{-1}$  there was one intensity measurement point. Choosing a small resolution aimed to make the peak clearly visible, because the smaller the resolution the more visible the peak.

The infrared spectrum of *C. aeruginosa* rhizome essential oil was taken from five geographically different regions (Figure 2), namely Kaloran, Kalibakung, Jumantoro, Bayat, and Trucuk. The fingerprint area at wave numbers 1750-700  $\text{cm}^{-1}$  showed a similar spectrum pattern, but there were differences in each absorption value due to the content or concentration of compounds in each essential oil of the *C. aeruginosa* rhizome. The essential oil of *C. aeruginosa* rhizomes had varying absorption bands: band 1 (3000-2750  $\text{cm}^{-1}$ ) showing the CH group (methyl or methylene) in the aliphatic chain in the essential oil; band 2 (around 1725-1715  $\text{cm}^{-1}$ ) showing the

C=O (ketone) group; band 3 (1600-1650  $\text{cm}^{-1}$ ) showing C=C (alkene); and band 4 (1300-1000  $\text{cm}^{-1}$ ) indicating the presence of stretching vibrations of CO in compounds containing ether or alcohol. These bands (Figure 2) showed that they contained the same contents of chemical compounds but with different levels.

PCA is a statistical technique applied to a set of variables, in this case the wave number in the essential oil of *C. aeruginosa* rhizomes from five different regions to determine the relationships or similarities between one test sample and another. The PCA method was applied using *metaboanalysis 6.0*, resulting in a number of data in the form of score plots and biplots. Score plot was used to present a plot of the relationship or similarity between the essential oils of *C. aeruginosa* rhizomes from the five regions based on the Principal Component (PC) values. In this study, two PCs were used, namely PC1 and PC2, which were marked with points that had a certain distance between one point and another. Regarding PC, objects with almost the same or close PC values can be said to have similar physicochemical properties. Therefore, PCA can be used for object grouping purposes<sup>[19]</sup>.

A biplot is a combination of score plot and loading plot. The loading plot shows how strongly each variable influences the PC by depicting it as a vector. If two vectors are forming an angle of less than  $90^\circ$ , then the two variables have a positive correlation. If it forms a right angle or around  $90^\circ$ , the two variables can't correlate. If they form an angle greater than  $90^\circ$  to  $180^\circ$ , then the two variables show a negative correlation<sup>[20]</sup>.

#### PCA of *C. aeruginosa* essential oil

In Figure 3, the highest eigenvalue results were shown in PC1 and then PC2, which means that these values contributed to the compound content of 59.8% on PC1 and 24.5% on PC2. In this analysis, PC1 and PC2 can be used to represent 100% of the variables (cumulative).

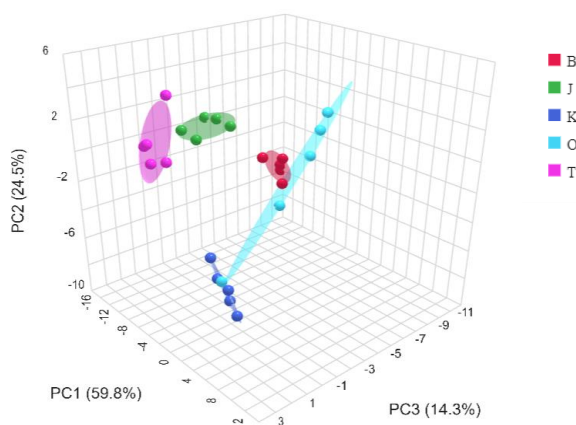


**Figure 3. Results *Principal Component Analysis* (PCA)**

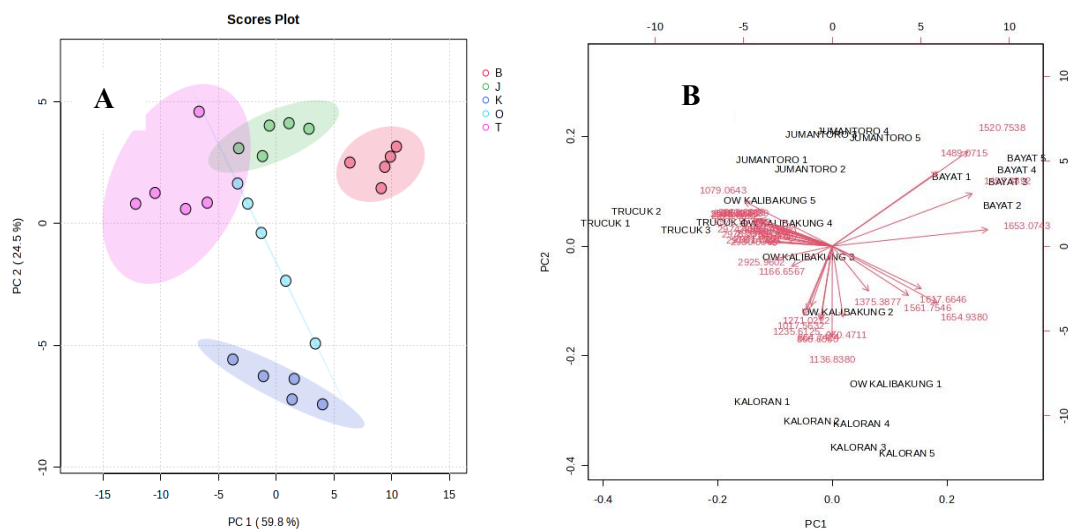


**Figure 4. PCA results: (A) Score plot ; (B) Biplot on FTIR**

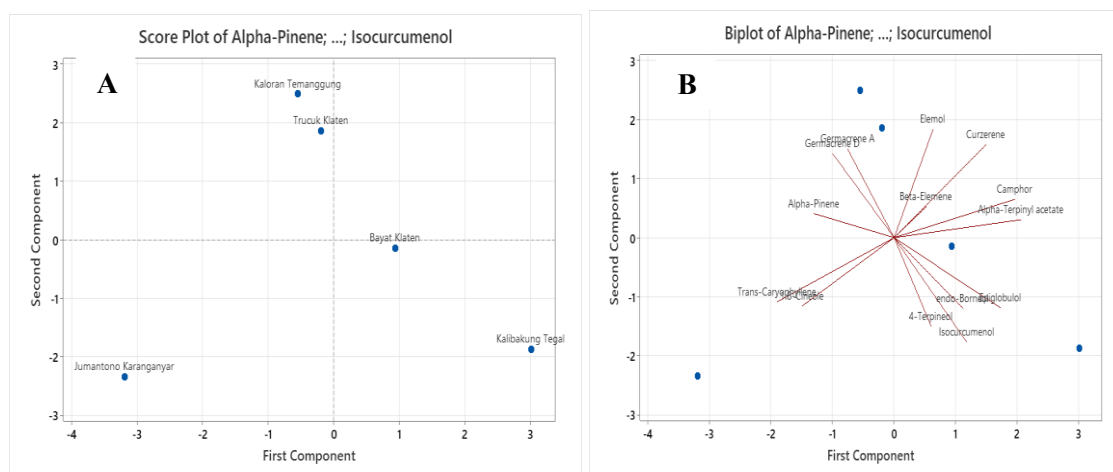


**Figure 5. PCA results: (A) Score plot ; (B) Biplot on GC-MS**

The relationship between the five essential oils of *C. aeruginosa* was not similar to one another, as seen by the score plot and biplot displayed in Figure 4. The score plot displays five points of essential oils from the five locations that were distant from one another. Conditions of the soil, temperature, and weather at the location of *C. aeruginosa* rhizomes grow are the causes of the dissimilarity. Because of this, the five rhizomes were different from one another in terms of the number of compounds contained, depending on the location in which they grew. The PCA results displayed in Figure 5 demonstrated a favorable association between curzerene and beta element compound's antibacterial and anti-inflammatory properties when compared to elemol and germacrene. A compound is considered to include essential oil components if its angle is smaller than 90°. The rhizome of *C. aeruginosa*, specifically beta-elemene, curzerene, elemol, and germacrene D, has antibacterial and antioxidant properties. Curzerene<sup>[21]</sup> and germacrene<sup>[22]</sup> have been studied for their antioxidant potential. According to Sieniawska et al. (2018), the beta-elemene in *Nigella damascena* has moderate to strong antibacterial activity against *M. tuberculosis*. The essential oil of *Neomirandea angularis* leaves contains *Bacillus cereus*, which is the target of Germacrene D's antibacterial activity<sup>[23]</sup>.

## PLS-DA

To obtain a high level of group separation and improve variable recognition for sample classification, the PLS-DA technique was used. 3D PLS-DA obtained on the sample distribution (Figure 6) based on the wave numbers identified in the essential oil of *C. aeruginosa* rhizomes showed that all the test samples could be clearly classified into five groups according to their origin.

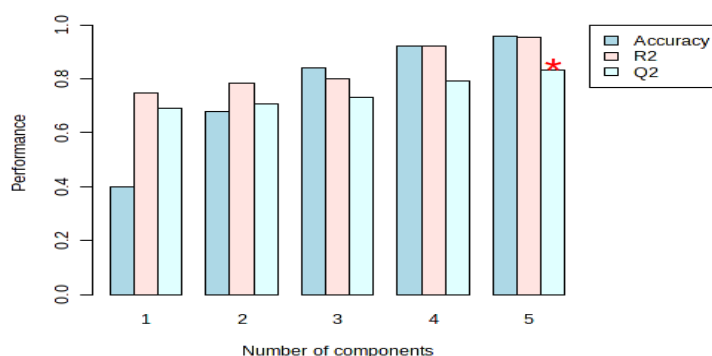


**Figure 6. R2 and Q2 intercept values from permutations in PLS-DA on FTIR**

The main purpose of permutations in PLS-DA is to test the significance of the model that has been built and evaluate the model. R-squared ( $R^2$ ) and Q-squared ( $Q^2$ ) are evaluation parameters that are often used in the context of discriminant principal component analysis with the PLS-DA method. However, the  $R^2$  and  $Q^2$  values are not obtained directly from permutations, but are more often used in internal cross-validation of PLS-DA models.  $R^2$  on PC2 was 0.79, where the higher the value, the better the model at explaining variations in response data, while  $Q^2$  on PC2 was 0.70, which was obtained from evaluation through cross validation; the higher the value, the better the model to predict new data. Some studies use specific significance levels (e.g.,  $\alpha = 0.05$ ) to determine whether  $R^2$  and  $Q^2$  values are considered significant. That is, if the original values are higher or lower than the values resulting from permutation at the selected significance level, then the model is considered significant. The above results indicated that the PLS-DA technique would be more suitable than PCA to provide visual differentiation of test samples according to origins regarding quality control.

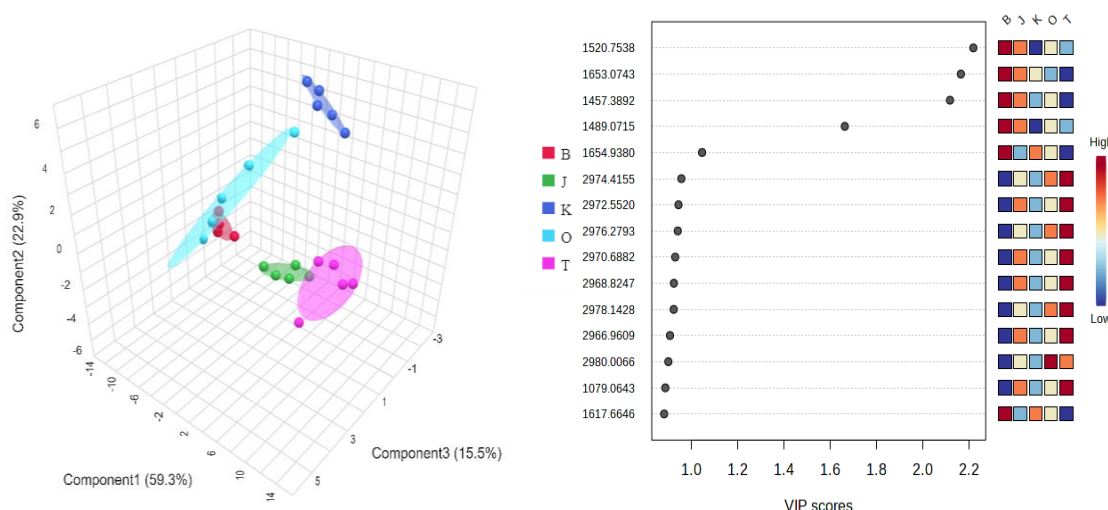


**Figure 7. PLS-DA results and VIP values on FTIR**

Based on PLS-DA, the loading plot shows the contribution of each to the sample discrimination variable. Based on VIP values in Figure 7, some wave numbers had VIP values  $> 1.5$ , so it can be concluded that wave number  $g$  of 1489.0715; 1475.3892; 1520.7538; and 1653.0743  $\text{cm}^{-1}$  were the most important wave numbers in showing differences in *C. aeruginosa* rhizome essential oil.

## CONCLUSION

*C. aeruginosa* rhizome is a traditional herb that is widely used in many countries. In this study, an efficient strategy was proposed for quality control of *C. aeruginosa* rhizomes as raw materials in the pharmaceutical and natural products industry. Even though there were variations in the chemical contents due to differences in geographical origins, the GC-MS fingerprint profiles of the essential oil samples were similar based on visual inspection, making it difficult to classify the essential oil samples according to their different origins. Therefore, the chemometric technique was applied with the FTIR fingerprint technique for recognition of *C. aeruginosa* rhizomes from various origins. It has shown that some techniques such as PCA and PLS-DA were capable of classifying the samples objectively. Furthermore, four wave numbers were found to be the most important variables in differentiating *C. aeruginosa* rhizomes for the quality control of *C. aeruginosa* rhizomes using quantitative analysis.

## Conflict of Interest

*The authors have declared no conflict of interest*

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## REFERENCES

1. Sun W, Wang S, Zhao W, et al. Chemical constituents and biological research on plants in the genus *Curcuma*. *Crit Rev Food Sci Nutr*. 2017;57(7):1451-1523. doi:10.1080/10408398.2016.1176554
2. Yuandani, Jantan I, Rohani AS, Sumantri IB. Immunomodulatory Effects and Mechanisms of *Curcuma* Species and Their Bioactive Compounds: A Review. *Front Pharmacol*. 2021;12. doi:10.3389/fphar.2021.643119
3. Xiang Z, Wang XQ, Caib XJ, Zenga S. Metabolomics study on quality control and discrimination of three *curcuma* species based on gas chromatograph-mass spectrometry. *Phytochem Anal*. 2011;22(5):411-418. doi:10.1002/pca.1296
4. Sirat HM, Jamil S, Hussain J. Essential oil of *curcuma aeruginosa* roxb. From Malaysia. *J Essent Oil Res*. 1998;10(4):453-458. doi:10.1080/10412905.1998.9700942
5. Theanphong O, Kirdmanee C. *Chemical Constituents and Biological Activities of Essential Oil From Curcuma aeruginosa Roxb. Rhizome Biological Activities of Zingiberaceous Plants View Project.*; 2015. <https://www.researchgate.net/publication/301215545>
6. Aziz JA, Saidi NB, Ridzuan R, et al. Chemical Profiling of *Curcuma aeruginosa* Roxb. Essential Oil and their Antimicrobial Activity against Pathogenic Microbes. *J Essent Oil-Bearing Plants*. 2021;24(5):1059-1071. doi:10.1080/0972060X.2021.1971570
7. Wahyuni DSC, Wardianti MP, Rinanto Y, Marliyana SD. Metabolite Profiling of Three *Curcuma* Species (*Zingiberaceae*) Based on H-NMR Spectroscopy. *ALCHEMY J Penelit Kim*. 2019;15(1):79.

doi:10.20961/alchemy.15.1.23914.79-88

8. Popa RM, Fetea F, Socaciu C. Atr-ftir-mir spectrometry and pattern recognition of bioactive volatiles in oily versus microencapsulated food supplements: Authenticity, quality, and stability. *Molecules*. 2021;26(16). doi:10.3390/molecules26164837
9. Truzzi E, Marchetti L, Bertelli D, Benvenuti S. Attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy coupled with chemometric analysis for detection and quantification of adulteration in lavender and citronella essential oils. *Phytochem Anal*. 2021;32(6):907-920. doi:10.1002/pca.3034
10. Chun MH, Kim EK, Yu SM, et al. GC/MS combined with chemometrics methods for quality control of *Schizonepeta tenuifolia* Briq: Determination of essential oils. *Microchem J*. 2011;97(2):274-281. doi:10.1016/j.microc.2010.09.015
11. Ahmad I, Fikri JAN, Arifianti AE, Abdullah S, Mun'im A. The combination of ATR-FTIR and chemometrics for rapid analysis of essential oil from Myrtaceae plants – A review. *J Appl Pharm Sci*. 2022;12(6):030-042. doi:10.7324/JAPS.2022.120604
12. Syafri S, Jaswir I, Yusof F, Rohman A, Ahda M, Hamidi D. The use of instrumental technique and chemometrics for essential oil authentication: A review. *Results Chem*. 2022;4. doi:10.1016/j.rechem.2022.100622
13. Ruiz-Perez D, Guan H, Madhivanan P, Mathee K, Narasimhan G. So you think you can PLS-DA? *BMC Bioinformatics*. 2020;21. doi:10.1186/s12859-019-3310-7
14. Angel G. Isolation and evaluation of bioactive non starch polysaccharides from eight starchy *Curcuma* species. *Nat Prod J*. 2013;3(3):204-211. doi:10.2174/22103155113039990009
15. Hirri A, Bassbasi M, Platikanov S, Tauler R, Oussama A. FTIR Spectroscopy and PLS-DA Classification and Prediction of Four Commercial Grade Virgin Olive Oils from Morocco. *Food Anal Methods*. 2016;9(4):974-981. doi:10.1007/s12161-015-0255-y
16. Agatonovic-Kustrin S, Ristivojevic P, Gegechkori V, Litvinova TM, Morton DW. Essential oil quality and purity evaluation via ft-ir spectroscopy and pattern recognition techniques. *Appl Sci*. 2020;10(20):1-12. doi:10.3390/app10207294
17. Ministry of Health. *Indonesian Herbal Pharmacopeia II Edition 2017 Ministry Health of The Republic of Indonesia 615.1 Ind F.*; 2017.
18. Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. *Nutrients*. 2018;10(9). doi:10.3390/nu10091196
19. Riyanta AB, Riyanto S, Lukitaningsih E, Rohman A. The employment of fourier transform infrared spectroscopy (FTIR) and chemometrics for analysis of candlenut oil in binary mixture with grape seed oil. *Food Res*. 2020;4(1):184-190. doi:10.26656/fr.2017.4(1).279
20. Widodo H, Sismindari S, Asmara W, Rohman A. Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *J Appl Pharm Sci*. 2019;9(6):99-105. doi:10.7324/JAPS.2019.90614
21. da Costa JS, Barroso AS, Mourão RH V., da Silva JKR, Maia JGS, Figueiredo PLB. Seasonal and antioxidant evaluation of essential oil from *Eugenia uniflora* L., curzerene-rich, thermally produced in situ. *Biomolecules*. 2020;10(2). doi:10.3390/biom10020328
22. Casiglia S, Bruno M, Bramucci M, et al. *Kundmannia sicula* (L.) DC: a rich source of germacrene D. *J Essent Oil Res*. 2017;29(6):437-442. doi:10.1080/10412905.2017.1338625
23. Washington VD, Palazzo MC, Haber WA, Setzer WN. The chemical composition and antibacterial activity of the leaf essential oil of *neomirandea angularis* (asteraceae) from monteverde, Costa Rica. *J Essent Oil-Bearing Plants*. 2010;13(1):108-111. doi:10.1080/0972060X.2010.10643797

## ORIGINAL ARTICLE

# Fourier Transform Infrared Spectroscopy combined with chemometrics for quality control of *Curcuma aeruginosa* rhizomes: An essential oil analysis

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## ABSTRACT

Rhizome of *Curcuma aeruginosa* is a traditional herb utilized in many nations. The compound content in *C. aeruginosa* rhizomes can vary depending on their geographical origin. This study aims to determine the chemical fingerprints for quality control and identify the active ingredients in *C. aeruginosa* essential oil (CAEO) based on multiple geographic origins. Chemical fingerprints of CAEO from five different geographic origins have been determined using Fourier Transform Infrared Spectroscopy (FTIR). Principal component analysis and partial least squares discriminant analysis were utilized to examine the chemical data of CAEO utilizing pattern recognition techniques. A total of 52 fluctuating compounds were identified in gas chromatography-mass spectra instrumentation, and the highest essential oil result was the 1,8-cineole compound content. According to the results from the FTIR analysis, CAEO samples were successfully grouped based on their geographical origins. The experimental results showed that the wave numbers 1489.0715, 1475.3882, 1520.7538, and 1653.0743 per cm were used as markers to identify compounds in the CAEO based on the differences in the place of growth and quality control. This study showed that chemometric methods combined with FTIR-based fingerprinting could offer a dependable platform for verifying quality control and authentication.

**Key words:** *Curcuma aeruginosa*, chemometric, essential oil, Fourier Transform Infrared Spectroscopy, gas chromatography-mass spectra

## INTRODUCTION

Central Java is the largest traditional medicine business center in Indonesia, and it produces many medicinal plants from the Zingiberaceae family,<sup>1</sup> one of which is *Curcuma*

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*aeruginosa*. *C. aeruginosa* essential oil (CAEO) has potent antioxidant activity<sup>2,3</sup> and antimicrobial properties, and it is used in food preservation, pharmaceuticals, and natural therapy.<sup>4-6</sup> The essential oil content is influenced by several factors, namely genetics, environmental conditions, climate, geographic origin, and others.<sup>1,6</sup> Several studies from various regions state that the CAEO content in Malaysia is dominated by camphor (29.39%) and germacrene (21.21%) and in Thailand is dominated by curcumenol (38.70%) and  $\beta$ -pinene (27.50%). In India, it is dominated by curazerenone (32.21%) and 1,8-cineole (15.35%),<sup>7</sup> while

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in Nepal, it is dominated by curzerenone (59.60%) and germacrone (5.3%).<sup>14</sup> Therefore, it is very important to assess the differences in the quality and examine the chemical composition of CAEO based on geographic origin.

Currently, a number of chromatographic and spectroscopic methods have been widely used to control herbal medication quality. The chemical components in CAEO can be determined effectively using Fourier Transform Infrared Spectroscopy (FTIR) fingerprint technology combined with chemometrics.<sup>15-21</sup> The fingerprint method uses pattern recognition technology to identify chemical components in sample fingerprints. It may also be used as a quality criterion for herbs based on similarity values. Several studies on the chemical diversity of medicinal plants used a chemometric approach.<sup>22-24</sup> Therefore, to overcome this, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) are used to reduce the dimensions of the extensive data, grouping samples more accurately, but PLS-DA is more effective and relevant for multicollinearity problems.<sup>24</sup>

In chemometric data processing, samples from different origins can be classified efficiently, and the characteristic factors leading to this classification can be determined. This study uses FTIR applications combined with chemometrics to classify and analyze the CAEO using gas chromatography-mass spectra (GC-MS).

## MATERIALS AND METHODS

### Materials

The samples used in this research were rhizomes of *C. aeruginosa* from five regions, harvested in October 2022: Kaloran Temanggung; Kalibakung, Tegal; Jumantono, Karanganyar; Bayat, Klaten; and Trucuk, Klaten, while  $\text{Na}_2\text{SO}_4$  was obtained from SmartLab.

### Methods

#### Preparation of essential oils

Fresh rhizomes are sliced with an oil distillate obtained was and added with anhydrous  $\text{Na}_2\text{SO}_4$  to remove remaining water traces. Then, they were centrifuged at 4000 rpm for 5 min.<sup>25</sup>

#### Analysis of essential oil profiles using gas chromatography-mass spectra

##### Fourier Transform Infrared Spectroscopy analysis

FTIR spectra were obtained using a Cary 630 Agilent FTIR spectrophotometer (USA) equipped with an attenuated total reflectance (ATR) accessory crystal with a resolution of 8/cm at 32 scans. Spectra were scanned in the absorbance mode from 4000 to 650/cm, and the data were handled with

MicroLab Expert Software. Analyses were carried out at room temperature.<sup>26-28</sup>

### Chemometric

MetaBoAnalyst 6.0 software performed chemometry techniques such as PCA and PLS-DA. PCA is an unsupervised technique that uses class labels as a point of reference to find the most explanatory direction variance in a dataset. The PLS-DA technique is a regression extension of PCA, utilizing class information to maximize separation between observation groups.<sup>29</sup>

## RESULTS AND DISCUSSION

The distillation process was carried out to obtain the essential oils contained in the rhizomes of *C. aeruginosa*. The distillation was carried out using the steam-water distillation method because it can yield a lot of essential oils. According to the Indonesian Herbal Pharmacopoeia 2<sup>nd</sup> edition of 2017, the CAEO contain no less than 1.89%  $\gamma$ -l.<sup>30</sup> The *C. aeruginosa* rhizomes from the five areas had essential oil levels below 1.89%  $\gamma$ . This is because different soil types and heights of growing *C. aeruginosa* plants can affect oil yield. The results of the % essential oil yield of *C. aeruginosa* rhizome are shown in Table 2.

### Essential oil content of *Curcuma aeruginosa* rhizome

The GC-MS method was used to analyze the content of the CAEO. The essential oils in *C. aeruginosa* rhizomes originating from the five regions contained similar contents but with different levels. Such differences were influenced by the geographical conditions where the plants grew, such as weather, soil height, soil type, soil pH, temperature, and humidity, thus influencing the levels of plant metabolite content.<sup>31</sup> The essential oil content of *C. aeruginosa* rhizomes is shown in Table 3. Table 3 displays the 52 peaks that accounted for 98.04%–99.76% of the volatile compounds. However, only 8–12 major compounds were identified, with a more than 2% peak area. The 1,8-cineole compound was found to have the highest level in the essential oils from the five regions. The camphor and isocurcumenol

**Table 1: Gas chromatography-mass spectra chromatography conditions**

| Condition                         | Setting value                              |
|-----------------------------------|--------------------------------------------|
| GC instrument                     | GC-MS-QP 2010 SE (Shimadzu, Japan)         |
| Column/oven temp                  | Agilent <sup>®</sup> capillary column/75°C |
| Injection temperature (°C)        | 175                                        |
| Flow rate of carrier gas (mL/min) | He at 0.8                                  |
| Pressure (kPa)                    | 45.9                                       |
| Total flow (mL/min)               | 83.8                                       |
| Linear velocity (cm/s)            | 32.9                                       |
| Purge flow (mL/min)               | 3.0                                        |
| Split ratio                       | 100.0                                      |
| Ion source temperature (°C)       | 200                                        |
| Interface temperature (°C)        | 225                                        |

GC: Gas chromatography; GC-MS: GC-mass spectra

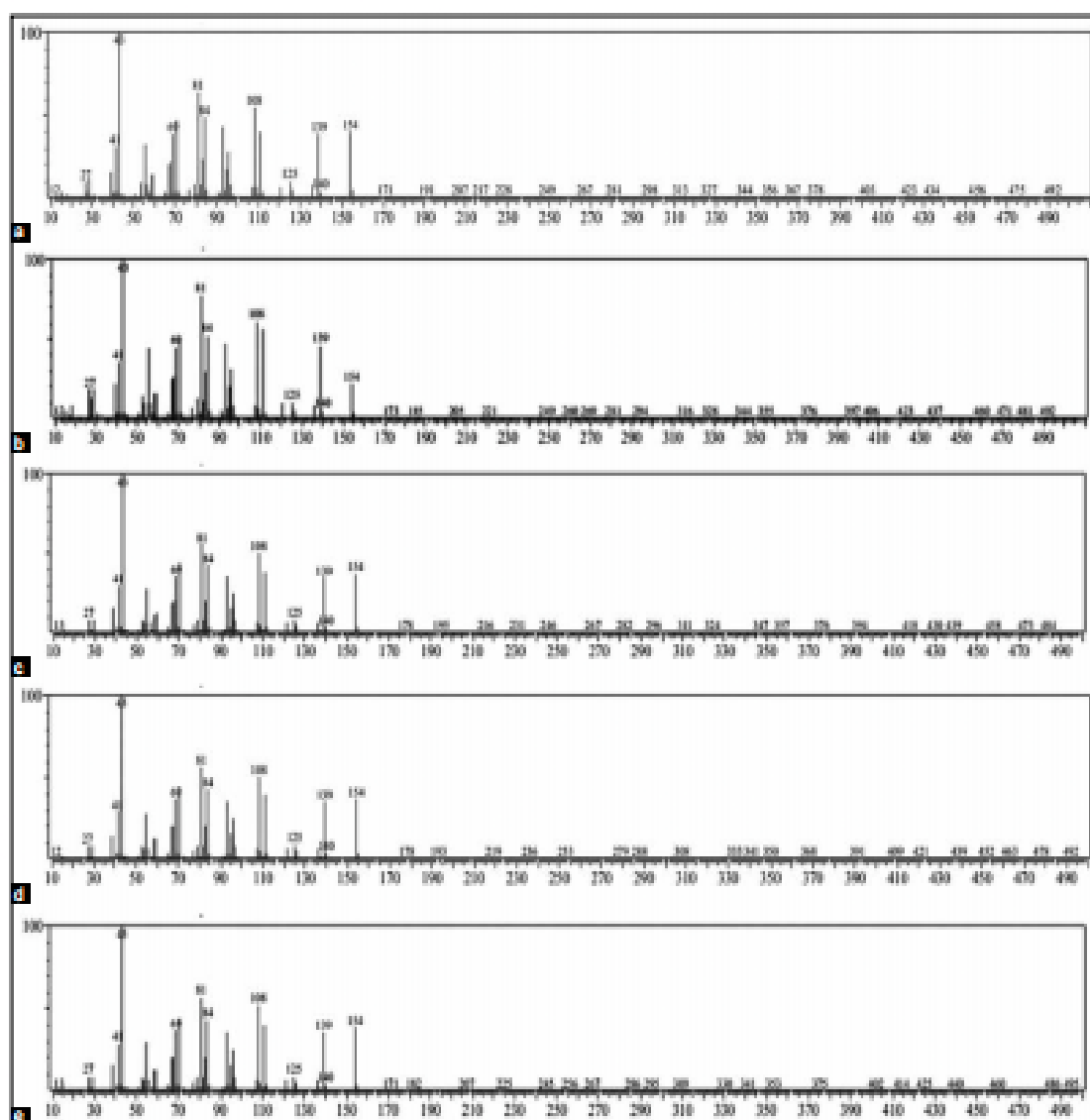


**Figure 1:** Representative chromatogram of *Curcuma aeruginosa* rhizome essential oil from different origins: (a) Kalaran, (b) Kalibakung, (c) Jumantono, (d) Bayat, (e) Trucuk

**Table 2: Percentage yield *Curcuma aeruginosa* rhizome essential oil**

| Number | Area                    | Yield (%) |
|--------|-------------------------|-----------|
| 1      | Kalaran - Temanggung    | 0.108     |
| 2      | Kalibakung - Tegal      | 0.104     |
| 3      | Jumantono - Karanganyar | 0.105     |
| 4      | Bayat - Klaten          | 0.079     |
| 5      | Trucuk - Klaten         | 0.120     |

compounds had different essential oil compositions among the five regions. Variations in the chemical composition of essential oils may be caused by differences in geographical

origins and environmental conditions, leading to differences in the quality of *C. aeruginosa* rhizome.

The compound 1,8-cineole is a compound contained in CAEO that is thought to be an inhibitor of Wnt/ $\beta$ -catenin activity in HNSCC through decreased GSK-3 inhibition, which leads to decreased levels of WNT1<sup>[20]</sup> and inhibiting the growth of MCF-7 cancer cells<sup>[21]</sup> likewise as an antibacterial<sup>[22]</sup> and anti-inflammatory.<sup>[23]</sup> Another compound also identified in the CAEO is camphor, capable of inhibiting the cell cycle and inducing apoptosis through the mitochondrial pathway in HCT-116 cells *in vitro* by 30% and then stopping cell proliferation.<sup>[24]</sup> The use of camphor relieves pain, itchiness, and irritation in

Table 3: Essential oil content of *Curcuma aeruginosa* rhizomes (2 replications)

| Compound                                                                            | Molecular weight  | Concentration (%)     |                    |                            |               |                |
|-------------------------------------------------------------------------------------|-------------------|-----------------------|--------------------|----------------------------|---------------|----------------|
|                                                                                     |                   | Kuloran, Teman gunung | Kulibakung, Tegall | Juman tonsa, Kuran gan yar | Bayat, Kliten | Trucuk, Kliten |
| Alpha-pinene                                                                        | $C_{10}H_{16}$    | 0.35                  | 0.24               | 0.55                       | 0.53          | 0.65           |
| Camphene                                                                            | $C_{10}H_{16}$    | 0.93                  | 0.73               | 1.21                       | -             | 1.58           |
| Beta-pinene                                                                         | $C_{10}H_{16}$    | 1.28                  | -                  | 1.64                       | 1.52          | 1.52           |
| Beta-myrcene                                                                        | $C_{10}H_{16}$    | -                     | -                  | 0.21                       | 0.16          | 0.19           |
| 1,8-diacetate                                                                       | $C_{15}H_{24}O$   | 26.66                 | 28.38              | 34.01                      | 29.35         | 30.93          |
| Camphor                                                                             | $C_{15}H_{24}O$   | 11.47                 | 19.05              | 3.24                       | 18.37         | 18.86          |
| 2-undecanol                                                                         | $C_{11}H_{22}O$   | -                     | 0.47               | 0.46                       | -             | -              |
| Endo-borneol                                                                        | $C_{15}H_{26}O$   | 0.70                  | 1.09               | 0.91                       | 0.92          | 1.00           |
| 4-terpineol                                                                         | $C_{15}H_{26}O$   | 0.46                  | 1.00               | 0.89                       | 0.82          | 0.90           |
| Alpha-terpinyl acetate                                                              | $C_{17}H_{30}O_2$ | 0.74                  | 1.75               | 0.16                       | 1.41          | 1.50           |
| Beta-dimians                                                                        | $C_{15}H_{24}$    | 0.56                  | 0.49               | 0.48                       | 0.85          | 0.58           |
| Trans-Caryophyllene                                                                 | $C_{15}H_{24}$    | 0.24                  | 0.18               | 0.48                       | 0.30          | 0.18           |
| Beta-farnesene                                                                      | $C_{15}H_{24}$    | -                     | -                  | 0.28                       | 0.27          | 0.20           |
| Alpha-humulene                                                                      | $C_{15}H_{24}$    | 1.83                  | -                  | -                          | 0.21          | -              |
| Germacrene D                                                                        | $C_{15}H_{24}$    | 0.22                  | 0.18               | 0.21                       | 0.19          | 0.26           |
| Beta-selinene                                                                       | $C_{15}H_{24}$    | -                     | 0.15               | -                          | 0.14          | 0.17           |
| Curazene                                                                            | $C_{15}H_{24}O$   | 0.43                  | 0.41               | 0.35                       | 0.41          | 0.41           |
| Germacrene A                                                                        | $C_{15}H_{24}$    | 0.17                  | 0.15               | 0.16                       | 0.15          | 0.19           |
| Germacrene B                                                                        | $C_{15}H_{24}$    | 0.17                  | -                  | 0.15                       | -             | 0.18           |
| Epiglobulol                                                                         | $C_{15}H_{24}O$   | 0.40                  | 0.46               | 0.40                       | 0.41          | 0.39           |
| Elemol                                                                              | $C_{15}H_{24}O$   | 0.29                  | 0.26               | 0.22                       | 0.24          | 0.30           |
| Boldione                                                                            | $C_{15}H_{24}O_2$ | 19.06                 | -                  | 14.97                      | 14.58         | 13.90          |
| Isocoumarol                                                                         | $C_{15}H_{24}O_2$ | 2.86                  | 8.89               | 5.97                       | 5.95          | 4.68           |
| Juniper camphor                                                                     | $C_{15}H_{24}O$   | -                     | -                  | 1.47                       | 1.98          | 1.94           |
| 1-(10), 4-aromadene diatriene                                                       | $C_{15}H_{24}$    | -                     | -                  | 0.51                       | 0.95          | 0.54           |
| Spatulanol                                                                          | $C_{15}H_{24}O$   | -                     | -                  | 1.08                       | 1.44          | 0.15           |
| Germacrene                                                                          | $C_{15}H_{24}O$   | -                     | 3.38               | 3.14                       | 3.62          | 3.45           |
| Aromadendrene epoxide                                                               | $C_{15}H_{24}O$   | -                     | -                  | 1.84                       | 0.48          | 0.50           |
| Trans-longiplocaradiol                                                              | $C_{15}H_{24}O$   | -                     | 0.41               | 0.57                       | -             | -              |
| Germacord                                                                           | $C_{15}H_{24}O$   | -                     | -                  | 3.42                       | -             | -              |
| 3-methyl-5-(2,6,6-trimethyl-1-cyclohexene-1-yl)-1-pentyl-3-d                        | $C_{21}H_{36}O$   | 1.44                  | 1.73               | -                          | -             | 0.45           |
| (E)-2,3,3-trimethyl-2-(3"-methyl-1"-3"-butadiene-1"-yl)-6-methylidene cyclohexanone | $C_{21}H_{36}O$   | 3.27                  | 2.97               | -                          | -             | 2.48           |
| Trihydrol(5,1,0,0 (3,5))octane-2,6-dione, 1,3,5,7-tetramethyl-                      | $C_{21}H_{36}O_2$ | -                     | -                  | 3.69                       | 3.49          | -              |
| Cyclohexane, 1,1-dimethyl-2,4-bis (1-methyl ethenyl)-, cis-                         | $C_{10}H_{18}$    | 5.16                  | -                  | 3.84                       | -             | -              |
| 2-cyclohexen-1-one, 4-ethyl-4-hydroxy-3,5,5-trimethyl-                              | $C_{14}H_{20}O$   | -                     | 15.95              | -                          | -             | -              |
| 3,7-cyclododecadien-1-one, 10-(1-methyl ethenyl)-, (E,E)                            | $C_{12}H_{20}O$   | 3.15                  | -                  | -                          | -             | -              |

Contd..

Table 3: Contd...

| Compound                                                                                                                                                  | Molecular weight  | Kalo min, Tieman ggung | Concentration (%) |                             |                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|------------------------|-------------------|-----------------------------|-----------------|
|                                                                                                                                                           |                   |                        | Kalibakung, Tegul | Jumari tonso, Kurangan gung | Trucuk, Klanten |
| Dihydrocarone                                                                                                                                             | $C_{17}H_{16}O$   | -                      | 4.01              | -                           | 4.47            |
| Calarene                                                                                                                                                  | $C_{17}H_{16}$    | 5.39                   | -                 | 3.33                        | 3.98            |
| 8icyd(2,2,2)Octa-2,5-Diene, 1,2,3,6-tetramethyl                                                                                                           | $C_{10}H_{16}$    | -                      | 4.70              | -                           | -               |
| Lactonoxide(0)                                                                                                                                            | $C_{10}H_{16}O$   | 0.96                   | 0.86              | 0.72                        | 1.11            |
| Cundione                                                                                                                                                  | $C_{11}H_{16}O_2$ | 2.76                   | -                 | -                           | -               |
| Lutane                                                                                                                                                    | $C_{11}H_{16}$    | -                      | -                 | 2.84                        | -               |
| 1H-cyclopropylidene phtisole, 1A,2,4,5,6,7,7A,7B-octa hydro- 1,1,7,7A, (E)-2,3,3-trimethyl-2,3'-methyl-1',3'-butadien-1'-yl-(6-methyl)ide noid oheva none | $C_{17}H_{20}O$   | -                      | -                 | 3.59                        | -               |
| Tricyd(5,2,2,0 (1,6)lunde can-3-ol, 2-methylene-6,8,8-trimethyl-                                                                                          | $C_{17}H_{20}O$   | 0.66                   | -                 | 2.32                        | 2.75            |
| (3E)-5-bispropyliden-6-methyl-3,6,9-decalen-2-one                                                                                                         | $C_{17}H_{20}O$   | 5.04                   | -                 | -                           | -               |
| Trans-p-menthyl-1 (7),8-dien-2-ol                                                                                                                         | $C_{17}H_{20}O$   | 0.89                   | -                 | -                           | -               |
| Hexadecane, 2,6,10,14-tetramethyl-                                                                                                                        | $C_{18}H_{36}$    | 0.42                   | -                 | -                           | -               |
| Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-7-octyl-                                                                                                     | $C_{24}H_{36}$    | 0.60                   | -                 | -                           | -               |
| Tetradecane, 1-chloro                                                                                                                                     | $C_{14}H_{28}Cl$  | 0.90                   | -                 | -                           | -               |
| Phenanthrene, 1,2,3,4,5,6,7,8-Octahydro-                                                                                                                  | $C_{19}H_{16}O$   | -                      | 0.22              | 0.17                        | 0.15            |
| Total Identified (%)                                                                                                                                      |                   | 99.46                  | 98.11             | 99.45                       | 98.04           |
| Monoterpene hydrocarbons (%)                                                                                                                              |                   | 4.39                   | 0.97              | 3.61                        | 2.42            |
| Oxygenated monoterpenes (%)                                                                                                                               |                   | 40.92                  | 71.70             | 39.66                       | 55.32           |
| Sesquiterpene hydrocarbons (%)                                                                                                                            |                   | 11.91                  | 1.15              | 18.19                       | 6.28            |
| Oxygenated sesquiterpenes (%)                                                                                                                             |                   | 37.05                  | 16.40             | 34.14                       | 27.15           |
| Oxygenated diterpenes (%)                                                                                                                                 |                   | 1.02                   | -                 | -                           | -               |
| Others (%)                                                                                                                                                |                   | 4.17                   | 7.89              | 3.86                        | 3.17            |

the affected area by activating particular transient receptor potential (TRP) channels, such as TRPV1, TRPV3, and TRPM8, and by inhibiting TRPA1. Camphor also produces a warm sensation and desensitizes sensory nerve endings.<sup>[9]</sup> Many pharmacological uses for camphor include expectorant, cough suppressant, rubefacient, antiseptic, antipruritic, anti-infective, anti-spasmodic, nasal decongestant, and cough suppression.<sup>[9]</sup>

#### Fourier Transform Infrared Spectroscopy analysis

Data for each *C. aeruginosa* rhizome essential oil sample were obtained by scanning the results with an FTIR using ATR. Scanning was performed in five replications in waves ranging from 4000 to 650 per cm. Wave number 1750–700 per cm was a fingerprint area with a resolution of 8/cm.

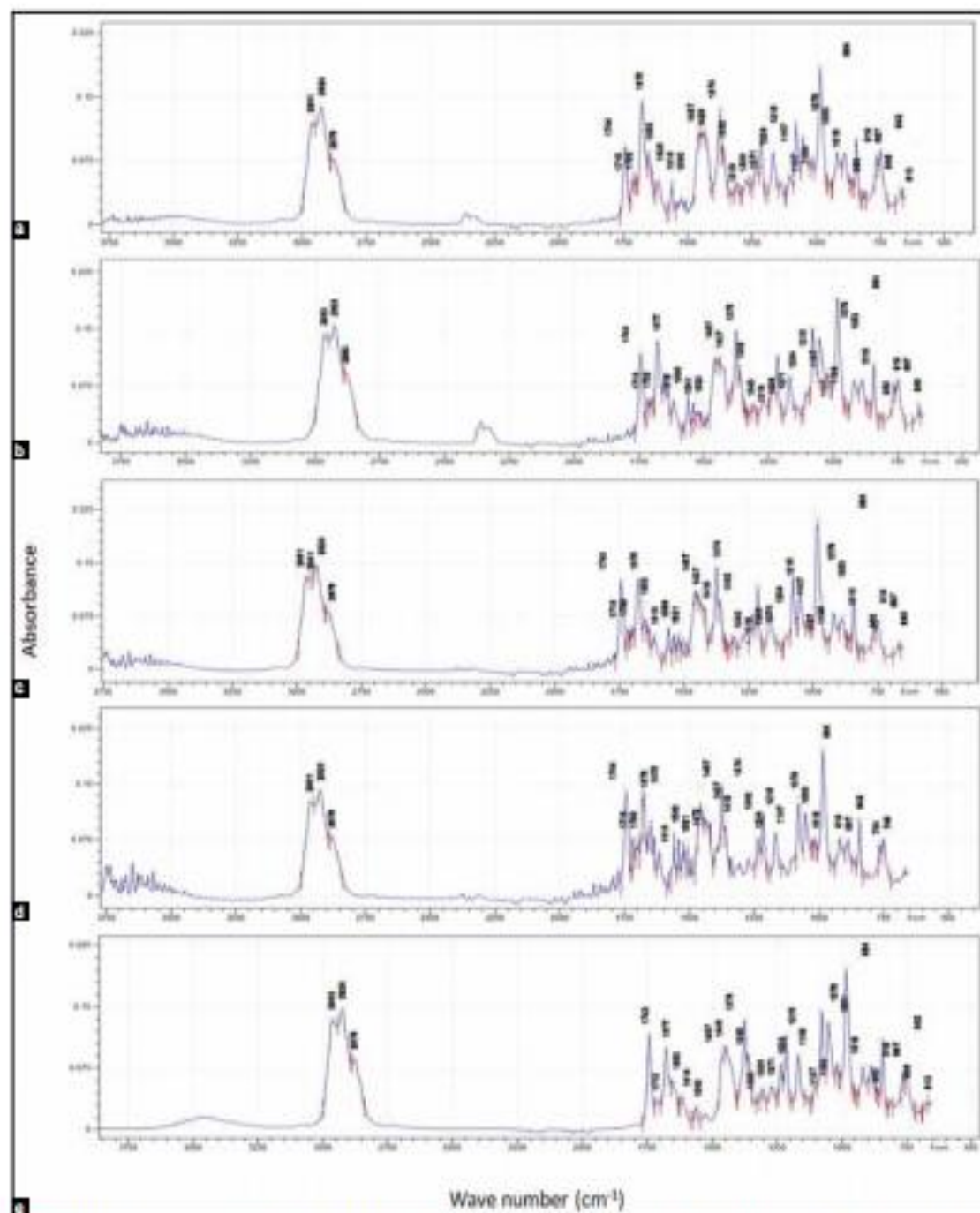


Figure 2: *C. aeruginosa* rhizome essential oil from different regions: (a) Caloran, (b) Kalihukung, (c) Jumantons, (d) Bayat, (e) Trucak

The infrared spectrum of CAEO was taken from five geographically different regions [Figure 2], namely Kaloran, Kalibakung, Jumanoro, Bayat, and Trucuk. The fingerprint area at wave numbers 1730–700 per cm showed a similar

spectrum pattern, but there were differences in each absorption value due to the content or concentration of compounds in each CAEO. The CAEO had varying absorption bands: band 1 (3000–2750 per cm) showing the CH group (methyl or methylene) in the aliphatic chain in the essential oil; band 2 (around 1725–1675 per cm) showing the C=O (ketone) group; band 3 (1650–1475 per cm) showing C=C (alkene); and band 4 (1300–1000 per cm), indicating the presence of stretching vibrations of CO in compounds containing ether or alcohol. These bands [Figure 2] showed that they contained the exact contents of chemical compounds but with different levels.

The PCA method was applied using *metaboanalysis 6.0*, resulting in a number of data in the form of score plots and biplots. A score plot was used to present a plot of the relationship or similarity between the CAEO from the five regions based on the principal component (PC) values. In this study, two PCs were used, namely PC1 and PC2, marked with points with a certain distance between one point and another. Regarding PC, objects with almost the same or close PC values can be said to have similar

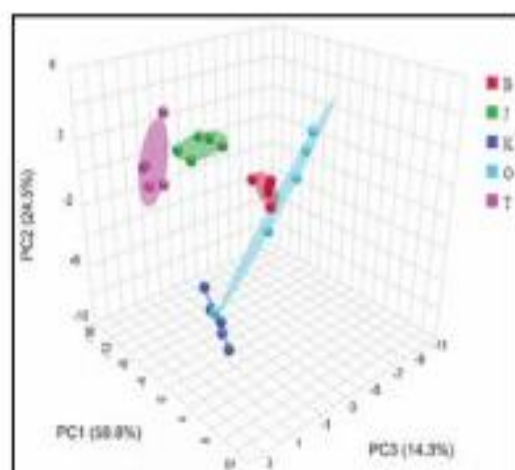


Figure 3: Results principal component analysis

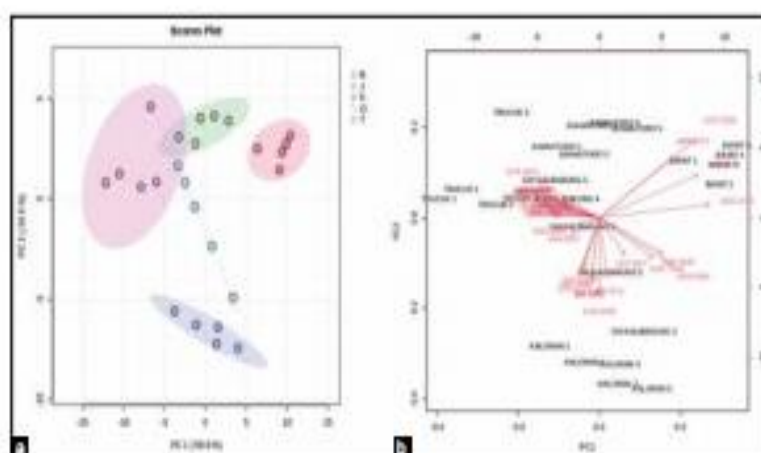


Figure 4: Principal component analysis results: (a) score plot; (b) biplot on Fourier Transform Infrared Spectroscopy

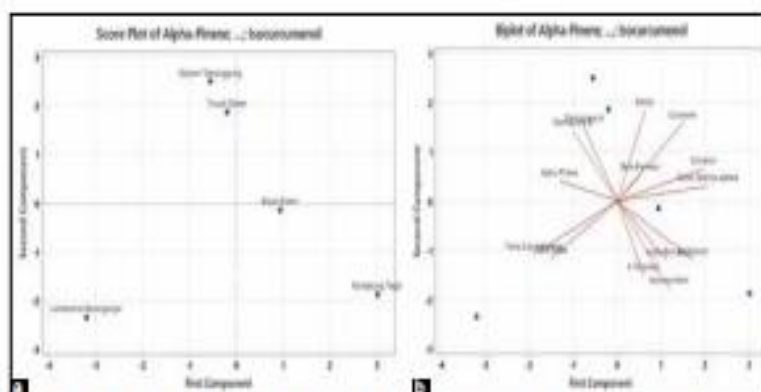


Figure 5: Principal component analysis results: (a) score plot; (b) biplot on gas chromatography-mass spectra

physicochemical properties. Therefore, PCA can be used for object grouping purposes.<sup>21</sup>

A biplot is a combination of a score plot and a loading plot. The loading plot shows how strongly each variable influences the PC by depicting it as a vector. If two vectors form an angle of  $<90^\circ$ , the two variables correlate positively. If it forms a right angle or around  $90^\circ$ , the two variables cannot correlate. The two variables show a negative correlation if they form an angle  $>90^\circ-180^\circ$ .<sup>24</sup>

### Principal component analysis of *Curcuma aeruginosa* essential oil

In Figure 3, the highest eigenvalue results were shown in PC1 and then PC2, which means that these values contributed to the compound content of 59.8% on PC1 and 24.5% on PC2. In this analysis, PC1 and PC2 can be used to represent 100% of the variables (cumulative).

The relationship between the five essential oils of *C. aeruginosa* was different, as seen by the score plot and biplot displayed in Figure 4. The score plot displays five points of essential

oils from the five locations distant from one another. The conditions of the soil, temperature, and weather at the location of *C. aeruginosa* rhizomes grow are the causes of the dissimilarity. Because of this, the five rhizomes differed in terms of the number of compounds contained, depending on the location in which they grew. The PCA results displayed in Figure 5 demonstrated a favorable association between curzerene and beta element compounds' antibacterial and anti-inflammatory properties when compared to elemol and germacrene. A compound is considered to include essential oil components if its angle is smaller than  $90^\circ$ . The rhizome of *C. aeruginosa*, specifically beta-element, curzerene, elemol, and germacrene D, has antibacterial and antioxidant properties. Curzerene<sup>25</sup> and germacrene<sup>26</sup> have been studied for their antioxidant potential. According to Sienkawska et al. (2018), the beta-elemene in *Nigella damascena* has moderate to strong antibacterial activity against *Mycobacterium tuberculosis*. The essential oil of *Nemorosus angulatus* leaves contains *Bacillus cereus*, which is the target of germacrene D's antibacterial activity.<sup>27</sup>

### Partial least squares discriminant analysis

To obtain a high level of group separation and improve variable recognition for sample classification, the PLS-DA technique was used. 3D PLS-DA obtained on the sample distribution (Figure 6) based on the wave numbers identified in the CABO showed that all the test samples could be clearly classified into five groups according to their origin.

The main purpose of permutations in PLS-DA is to test the significance of the model that has been built and evaluate the model.  $R^2$ -squared ( $R^2$ ) and  $Q^2$ -squared ( $Q^2$ ) are evaluation parameters often used in the context of discriminant PCA with the PLS-DA method. However, the  $R^2$  and  $Q^2$  values are not obtained directly from permutations but are often used in internal cross-validation of PLS-DA models.  $R^2$  on PC2 was 0.79, where the higher the value, the better the model at explaining variations in response data, while  $Q^2$  on

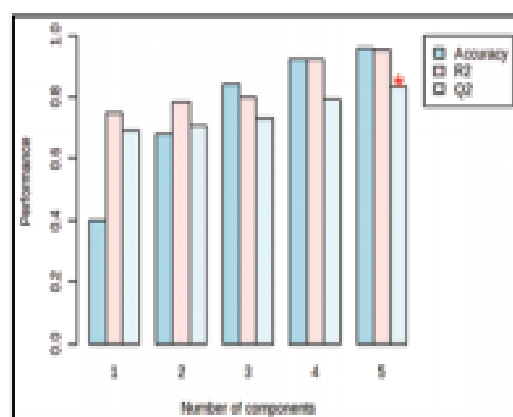


Figure 6:  $R^2$  and  $Q^2$  intercept values from permutations in partial least squares discriminant analysis on Fourier Transform Infrared Spectroscopy

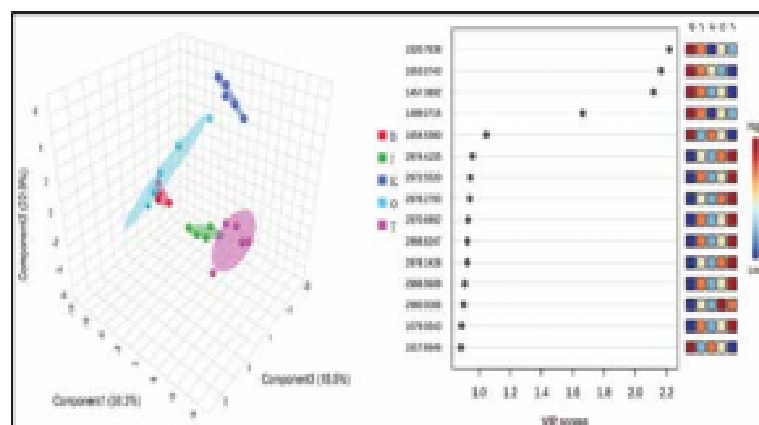


Figure 7: Partial least squares discriminant analysis results and VIP values on Fourier Transform Infrared Spectroscopy

PC2 was 0.70, which was obtained from evaluation through cross-validation; the higher the value, the better the model to predict new data. Some studies use specific significance levels (e.g.,  $\alpha=0.05$ ) to determine whether  $R^2$  and  $Q^2$  values are significant. If the original values are higher or lower than the values resulting from permutation at the selected significance level, the model is considered significant. The above results indicated that the PLS-DA technique would be more suitable than PCA to provide visual differentiation of test samples according to origins regarding quality control.

Based on PLS-DA, the loading plot shows the contribution of each to the sample discrimination variable. Based on VIP values in Figure 7, some wave numbers had VIP values  $>1.5$ , so it can be concluded that wave numbers of 1489.0715; 1475.3892; 1520.7538; and 1653.0743 per cm were the most important wave numbers in showing differences in *C. aeruginosa* rhizome essential oil.

## CONCLUSION

*C. aeruginosa* rhizome is a traditional herb widely used in many countries. This study proposed an efficient strategy for quality control of CAEO as raw materials in the pharmaceutical and natural products industry. Even though there were variations in the chemical contents due to differences in geographical origins, the GC-MS fingerprint profiles of the essential oil samples were similar based on visual inspection, making it difficult to classify the essential oil samples according to their different origins. Therefore, the chemometric technique was applied with the FTIR fingerprint technique to recognize CAEO from various origins. It has shown that some techniques, such as PCA and PLS-DA, could classify the samples objectively. Furthermore, four-wave numbers were the most important variables in differentiating *C. aeruginosa* rhizomes for the quality control of CAEO using quantitative analysis.

## Acknowledgment

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## Financial support and sponsorship

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Sari AP. Phytochemistry and biological activities of *Curcuma aeruginosa* (Roxb.). *Indones J Chem* 2022;22:576-98.
- Kamuceri TS, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma nanga* and *Zingiber cassumunar* from Malaysia. *Asian Pac J Trop Med* 2012;5:202-9.
- George M, Britto SJ. Phytochemical and antioxidant studies on the essential oil of The Rhizome of *Curcuma aeruginosa* Roxb. *Int Res J Pharm* 2015;6:573-9.
- Theanphong O, Mingranish W, Kirdmanee C. Chemical constituents and biological activities of essential from *Curcuma aeruginosa* Roxb. *Rhizoma*. Vol 13; 2015. Available from: <https://www.researchgate.net/publication/301215548>.
- Botabara I, Yunita D, Suparto IH. Antibacterial and biofilm degradation activity of extract from steam distillation residue of *Zingiberaceae* leaves against *Streptococcus mutans*. *J Indones Chem Soc* 2019;2:42.
- Mohammadhosseini M, Akbarzadeh A, Hashemi-Moghaddam H, Bahmanpour H, Shafagh A, Lotfi S. The relationship between chemical composition of the essential oils of *Platycladus orientalis* (L.) Franco and soils contamination in national oil company of Shahrzad, Iran. *J Essent Oil Bearing Plants* 2017;2(1):20-25.
- Dasoky NS, Satyal P, Setzer WN. Variations in the volatile compositions of curcuma species. *Food* 2019;8:777.
- Poudel DK, Ojha PK, Rokaya A, Satyal R, Satyal P, Setzer WN. Analysis of volatile constituents in curcuma species, viz. *C. aeruginosa*, *C. zataria* and *C. longa*, from Nepal. *Plants (Basel)* 2022;11:1932.
- Popa RM, Fetea F, Socaciu C. ATR-FTIR-MIR spectrometry and pattern recognition of bioactive volatiles in oily versus microencapsulated food supplements: Authenticity, Quality, and Stability. *Molecules* 2021;26:4837.
- Truzzi E, Marchetti L, Bertelli D, Benvenuti S. Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy coupled with chemometric analysis for detection and quantification of adulteration in lavender and citronella essential oils. *Phytochem Anal* 2021;32:907-20.
- Chuan MH, Kim EK, Yu SM, et al. GC/MS combined with chemometrics methods for quality control of *Schizandra leafy* Briq Determination of essential oils. *Micromech* [2011;9:274-81.
- Ahmad I, Fikri JA, Arifiani AE, Abdullah S, Mun'im A. The combination of ATR-FTIR and chemometrics for rapid analysis of essential oil from *Myrtaceae* plants - A review. *J Appl Pharm Sci* 2022;12:030-042.
- Syafri S, Jaswir I, Yusof F, Rohman A, Ahda M, Harimi D. The use of instrumental technique and chemometrics for essential oil authentication: A review. *Results Chem* 2022;4:777. [doi:10.1016/j.rechem.2022.100622].
- Ruiz-Perez D, Guan H, Madhivanan P, Mathew K, Namsirhan G. Soybean you can PLS-DA? *BMC Bioinformatics* 2020;21:2.
- Angel G. Isolation and evaluation of bioactive non starch polysaccharides from eight starchy *Curcuma* species. *Nat Prod* 2013;3:204-11.
- Hiri A, Boubou M, Ratikanov S, Izler R, Oussama A. FTIR spectroscopy and PLS-DA classification and prediction of four commercial grade virgin olive oils from morocco. *Food Methods* 2016;9:974-81.
- Agatonovic-Kustrin S, Risticovic P, Gageckori V, Litvinova TM, Morton DW. Essential oil quality and purity evaluation via IR spectroscopy and pattern recognition techniques. *Appl Sci* 2020;10:1-12.

18. Kemenkes. *Farmakope Herbal Indonesia Edisi II*. 2017. Kementerian Kesehatan Republik Indonesia 615.1 Ind F; 2017.
  19. Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. *Nutrients* 2018;10:1196.
  20. Rottger A, Bruchhage KL, Drenckhan M, Ploster-Martin K, Pries R, Wollenberg B. Inhibitory effect of 1,8-cineol on  $\beta$ -catenin regulation, WNT11 expression, and cellular progression in HNSCC. *Front Oncol* 2017;7:92.
  21. Fitria R, Sano DS, Priasetyanto BP, Hartanti, Nurcholis W. Volatile compound profiles and cytotoxicity in essential oils from the rhizome of *Curcuma ovaginosa* and *Curcuma zanthorrhiza*. *Biodiversitas* 2019;20:2943-8.
  22. Simsek M, Duman R. Investigation of effect of 1,8-cineol on antimicrobial activity of chlorhexidine gluconate. *Pharmacognosy Res* 2017;9:294-7.
  23. Jiang Z, Luo M, Ma W, Ma S, Wang Y, Zhang K. Protective effects of 1,8-cineole microcapsules against inflammation and gut microbiota imbalance associated weight loss induced by heat stress in broiler chicken. *Front Pharmacol* 2020;11:565945.
  24. Itani WS, El-Bara SH, Hassan SB, Lamson RL, Bazarbachi A, Gali-Muhtasib HU. Anti colon cancer components from Lebanese sage (*Salvia libanotica*) essential oil: Mechanistic basis. *Cancer Biol Ther* 2008;7:1768-73.
  25. Singh R, Jawaid T. *Cinnamomum camphora* (Kapur): Review. *Pharmacogn J* 2012;4:1-5.
  26. Hamidpour R, Hamidpour S, Hamidpour M, Hamidpour R. The Effect of Camphor Discovery for Treating Asthma Corresponding Author. Vol 2. Available from: <https://www.ojsonline.com>.
  27. Riyanto AB, Riyanto S, Lukitaningsih E, Rohman A. The employment of fourier transform infrared spectroscopy (FTIR) and chemometrics for analysis of candlenut oil in binary mixture with grape seed oil. *Food Res* 2020;4:184-90.
  28. Widodo H, Simandari S, Asmara W, Rohman A. Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *J Appl Pharm Sci* 2019;9:99-105.
  29. da Costa JS, Barroso AS, Mourão RH, da Silva JK, Maia JG, Figueiredo PL. Seasonal and antioxidant evaluation of essential oil from *Eugenia aniflora* L., carvacrol-rich, thermally produced *in vitro*. *Biomolecules* 2020;10:328.
  30. Casiglia S, Beano M, Benvenuti M, et al. *Kandaweria sicala* (L.) DC: A rich source of garmacene D. *J Essent Oil Res* 2017;28:437-42.
  31. Washington VD, Palazzo MC, Haber WA, Setzer WN. The chemical composition and antibacterial activity of the leaf essential oil of *Noniwood angulata* (Asteraceae) from Monteverde, Costa Rica. *J Essent Oil Bearing Plants* 2010;13:108-11.
- Author Queries ???
- AQ1: Kindly provide author's full name.
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- AQ4: Kindly cite Table 1 in the text part
- AQ5: Kindly check the edit. "," symbol changed in to decimal point. Kindly check and confirm.
- AQ6: Kindly provide author initial.
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- AQ11: Kindly check the unit throughout the article.
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- AQ13: Kindly check this author name does not match with the reference citation.
- AQ14: Kindly check the term and provide expansion if applicable.
- AQ15: Kindly cite Reference 18 in the text part.
- AQ16: Kindly provide the date, month, year on which this link was last accessed.
- AQ17: Kindly check the page number.
- AQ18: Please note we search this reference in Pub Med it was not found. Please check and provide six authors before et al.
- AQ19: Kindly provide the significance of "" in legend.
- AQ20: Please provide complete reference details such as page number.
- AQ21: Kindly provide complete reference details
- AQ22: Kindly check the placement of Figure 2,3,4,5 and 7

### Reply to reviewers' Comment file (JAPTR\_75\_24)

| Reviewer Number | Original comments of the reviewer                                                             | Reply by the author(s)                                                                                                                                                                                                                                                                                                                                                      | Changes done on page number and line number             |
|-----------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|
| AQ1             | Kindly provide author's full name                                                             | Purwanto Purwanto                                                                                                                                                                                                                                                                                                                                                           | Page number 1, line 11                                  |
| AQ2             | Kindly provide department name.                                                               | Doctoral Program in Pharmaceutical Science, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia                                                                                                                                                                                                                                                             | Page number 1, line 13                                  |
| AQ3             | Kindly provide English language.                                                              | <b>We still use that writing:</b><br><br>Universitas Gadjah Mada, Yogyakarta, Indonesia and Sekolah Tinggi Ilmu Farmasi (STIFAR) Yayasan Pharmasi, Semarang                                                                                                                                                                                                                 | Page number 1, line 14-15, 44                           |
| AQ4             | Kindly cite Table 1 in the text part                                                          | The GC-MS method (Table 1) was used to analyze the content of the CAEO. The essential oils in <i>C. aeruginosa</i> rhizomes originating from the five regions contained similar contents but with different levels                                                                                                                                                          | Page number 2, line 25                                  |
| AQ5             | Kindly check the edit. “,” symbol was changed into a decimal point. Kindly check and confirm. | Yes, that's right: “18.19”                                                                                                                                                                                                                                                                                                                                                  | Page number 5, line 11                                  |
| AQ6             | Kindly provide the author's initials.                                                         | Purwanto Purwanto<br><br>According to the journal's reflection, the author's name is written as Hartanti                                                                                                                                                                                                                                                                    | Page number 1, line 11<br><br>Page number 10, line 8    |
| AQ7             | Kindly review the sentence.                                                                   | Fresh rhizomes of <i>C. aeruginosa</i> were sliced with a thickness of approximately 1-2 mm, and then steamed and distilled for 4 hours over medium heat. The resulting distillate was separated from water by adding anhydrous Na <sub>2</sub> SO <sub>4</sub> . According to the Herbal Pharmacopoeia 2 <sup>nd</sup> edition, the CAEO must contain not less than 1.89%. | Page number 2, line 42<br><br>Page number 2, line 16-17 |
| AQ8             | Please provide complete manufacturer details such as company name, city, state and country.   | Spectra were scanned in the absorbance mode from 4000 to 650/cm, and the data were handled with MicroLab Expert Software developed by Bio-Rad Laboratories, headquartered in Hercules, California, USA.                                                                                                                                                                     | Page number 2, line 55                                  |
| AQ9             | Kindly check the heading.                                                                     | <b>RESULTS</b><br><br><b>DISCUSSION</b>                                                                                                                                                                                                                                                                                                                                     | Page number 2, line 10<br><br>Page number 7, line 13    |
| AQ10            | Kindly cite Figure 1 in the text part.                                                        | Variations in the chemical composition of essential oils may be caused by differences in geographical                                                                                                                                                                                                                                                                       | Page number 3, line 45                                  |

|      |                                                                                                                             |                                                                                                                                                                                                                                                                                |                         |
|------|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
|      |                                                                                                                             | origins and environmental conditions, leading to differences in the quality of <i>C. aeruginosa</i> rhizome (Figure 1)                                                                                                                                                         |                         |
| AQ11 | Kindly check the unit throughout the article.                                                                               | Not 1.89% v/w, but 1.89%                                                                                                                                                                                                                                                       | Page number 2, line 18  |
| AQ12 | Kindly check and confirm the phrase.                                                                                        | The PCA method was applied using using software Metaboanalyst 6.0, which is freely available at <a href="https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml">https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml</a> .                                        | Page number 7, line 14  |
| AQ13 | Kindly check this author name does not match with the reference citation.                                                   | Bibliography:<br><br>Sieniawska E, Sawicki R, Golus J, Swatko-Ossor M, Ginalska G, Skalicka-Wozniak K. <i>Nigella damascena</i> L. essential oil-a valuable source of $\beta$ -elemene for antimicrobial testing. <i>Molecules</i> . 2018;23(2). doi:10.3390/molecules23020256 | Page number 10, line 31 |
| AQ14 | Kindly check the term and provide expansion if applicable.                                                                  | Based on Variable Importance Projection (VIP) values in Figure 7                                                                                                                                                                                                               | Page number 9, line 14  |
| AQ15 | Kindly cite Reference 18 in the text part.                                                                                  | According to the Herbal Pharmacopoeia 2 <sup>nd</sup> edition, the CAEO must contain not less than 1.89% <sup>[18]</sup> .                                                                                                                                                     | Page number 2, line 18  |
| AQ16 | Kindly provide the date, month, year on which this link was last accessed.                                                  | Orawan T, Withawat M, Chalermopol K. Chemical constituents and biological activities of essential oil from <i>Curcuma aeruginosa</i> Roxb. Rhizome. <i>Bull Heal Sci Technol</i> . 2015;13(1):6-16.                                                                            | Page number 10, line 13 |
| AQ17 | Kindly check the page number.                                                                                               | Ahmad I, Fikri JA, Arifianti AE, Abdullah S, Mun'im A. The combination of ATR-FTIR and chemometrics for rapid analysis of essential oil from <i>Myrtaceae</i> plants – A review. <i>J Appl Pharm Sci</i> 2022;12:30-42                                                         | Page number 10, line 40 |
| AQ18 | Please note we search this reference in PubMed it was not found. Please check and provide six authors before <i>et al</i> . | Bibliography:<br><br>Sieniawska E, Sawicki R, Golus J, Swatko-Ossor M, Ginalska G, Skalicka-Wozniak K. <i>Nigella damascena</i> L. essential oil-a valuable source of $\beta$ -elemene for antimicrobial testing. <i>Molecules</i> . 2018;23(2). doi:10.3390/molecules23020256 | Page number 10, line 31 |

|      |                                                                |                                                                                                                                                                                                                          |                                                                                                                                                                                 |
|------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AQ19 | Kindly provide the significance of “*” in legend.              | “*” indicates that the model has excellent predictive performance, with $Q^2 \geq 0.7$ .                                                                                                                                 | Page number 8, line 38                                                                                                                                                          |
| AQ20 | Please provide complete reference details such as page number. | Syafri S, Jaswir I, Yusof F, Rohman A, Ahda M, Hamidi D. The use of instrumental technique and chemometrics for essential oil authentication: A review. Results Chem 2022;4: 1-10. [doi: 10.1016/j.rechem. 2022.100622]. | Page number 10, line 43                                                                                                                                                         |
| AQ21 | Kindly provide complete reference details                      | Ministry of Health of the Republic of Indonesia, “Indonesian Herbal Pharmacopoeia 2 <sup>nd</sup> Edition 2017, Ministry of Health of the Republic of Indonesia,” Indonesia, 2017.                                       | Page number 10, line 1                                                                                                                                                          |
| AQ22 | Kindly check the placement of Figure 2,3,4,5 and 7             | The placement of figures 2,3,4,5, and 7 is correct                                                                                                                                                                       | Figur 2: Page number 6, line 56<br>Figure 3: Page number 7, line 22<br>Figure 4: Page number 7, line 40<br>Figure 5: Page number 7, line 56<br>Figure 7: Page number 8, line 56 |

# Fourier Transform Infrared Spectroscopy combined with chemometrics for quality control of *Curcuma aeruginosa* rhizomes: An essential oil analysis

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*J. Adv. Pharm. Technol. Res.*

## ABSTRACT

Rhizome of *Curcuma aeruginosa* is a traditional herb utilized in many nations. The compound content in *C. aeruginosa* rhizomes can vary depending on their geographical origin. This study aims to determine the chemical fingerprints for quality control and identify the active ingredients in *C. aeruginosa* essential oil (CAEO) based on multiple geographic origins. Chemical fingerprints of CAEO from five different geographic origins have been determined using Fourier Transform Infrared Spectroscopy (FTIR). Principal component analysis and partial least squares discriminant analysis were utilized to examine the chemical data of CAEO utilizing pattern recognition techniques. A total of 52 fluctuating compounds were identified in gas chromatography-mass spectra instrumentation, and the highest essential oil result was the 1,8-cineole compound content. According to the results from the FTIR analysis, CAEO samples were successfully grouped based on their geographical origins. The experimental results showed that the wave numbers 1489.0715, 1475.3892, 1520.7538, and 1653.0743 per cm were used as markers to identify compounds in the CAEO based on the differences in the place of growth and quality control. This study showed that chemometric methods combined with FTIR-based fingerprinting could offer a dependable platform for verifying quality control and authentication.

**Key words:** *Curcuma aeruginosa*, chemometric, essential oil, Fourier Transform Infrared Spectroscopy, gas chromatography-mass spectra

## INTRODUCTION

Central Java is the largest traditional medicine business center in Indonesia, and it produces many medicinal plants from the Zingiberaceae family,<sup>[1]</sup> one of which is *Curcuma*

*aeruginosa*. *C. aeruginosa* essential oil (CAEO) has potent antioxidant activity<sup>[2,3]</sup> and antimicrobial properties, and it is used in food preservation, pharmaceuticals, and natural therapy.<sup>[4,5]</sup> The essential oil content is influenced by several factors, namely genetics, environmental conditions, climate, geographic origin, and others.<sup>[6]</sup> Several studies from various regions state that the CAEO content in Malaysia is dominated by camphor (29.39%) and germacrone (21.21%) and in Thailand is dominated by curcumenol (38.70%) and  $\beta$ -pinene (27.50%). In India, it is dominated by curzerenone (32.21%) and 1,8-cineole (15.35%),<sup>[7]</sup> while

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in Nepal, it is dominated by curzerenone (59.60%) and germacrone (5.3%).<sup>[8]</sup> Therefore, it is very important to assess the differences in the quality and examine the chemical composition of CAEO based on geographic origin.

Currently, a number of chromatographic and spectroscopic methods have been widely used to control herbal medication quality. The chemical components in CAEO can be determined effectively using Fourier Transform Infrared Spectroscopy (FTIR) fingerprint technology combined with chemometrics.<sup>[9,10]</sup> The fingerprint method uses pattern recognition technology to identify chemical components in sample fingerprints. It may also be used as a quality criterion for herbs based on similarity values. Several studies on the chemical diversity of medicinal plants used a chemometric approach.<sup>[11-13]</sup> Therefore, to overcome this, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) are used to reduce the dimensions of the extensive data, grouping samples more accurately, but PLS-DA is more effective and relevant for multicollinearity problems.<sup>[14]</sup>

In chemometric data processing, samples from different origins can be classified efficiently, and the characteristic factors leading to this classification can be determined. This study uses FTIR applications combined with chemometrics to classify and analyze the CAEO using gas chromatography-mass spectra (GC-MS).

## MATERIALS AND METHODS

### Materials

The samples used in this research were rhizomes of *C. aeruginosa* from five regions, harvested in October 2022: Kaloran Temanggung; Kalibakung, Tegal; Jumantono, Karanganyar; Bayat, Klaten; and Trucuk, Klaten, while Na<sub>2</sub>SO<sub>4</sub> was obtained from SmartLab.

### Methods

#### Preparation of essential oils

Fresh rhizomes of *C. aeruginosa* were sliced with a thickness of approximately 1-2 mm, and then steamed and distilled for 4 hours over medium heat. The resulting distillate was separated from water by adding anhydrous Na<sub>2</sub>SO<sub>4</sub>. Then, they were centrifuged at 4000 rpm for 5 min.<sup>[15]</sup>

#### Analysis of essential oil profiles using gas chromatography-mass spectra

##### Fourier Transform Infrared Spectroscopy analysis

FTIR spectra were obtained using a Cary 630 Agilent FTIR spectrophotometer (USA) equipped with an attenuated total reflectance (ATR) accessory crystal with a resolution of 8/cm at 32 scans. Spectra were scanned in the absorbance mode from 4000 to 650/cm, and the data were handled with MicroLab Expert (Bio-Rad Laboratories, headquartered in

Hercules, California, USA). Analyses were carried out at room temperature.<sup>[16,17]</sup>

### Chemometric

MetaboAnalyst 6.0 software performed chemometry techniques such as PCA and PLS-DA. PCA is an unsupervised technique that uses class labels as a point of reference to find the most explanatory direction variance in a dataset. The PLS-DA technique is a regression extension of PCA, utilizing class information to maximize separation between observation groups.<sup>[14]</sup>

## RESULTS AND DISCUSSION

The distillation process was carried out to obtain the essential oils contained in the rhizomes of *C. aeruginosa*. The distillation was carried out using the steam-water distillation method because it can yield a lot of essential oils. According to the Indonesian Herbal Pharmacopoeia 2<sup>nd</sup> edition, the CAEO must contain not less than 1.89%.<sup>[18]</sup> The *C. aeruginosa* rhizomes from the five areas had essential oil levels below 1.89%. This is because different soil types and heights of growing *C. aeruginosa* plants can affect oil yield. The results of the % essential oil yield of *C. aeruginosa* rhizome are shown in Table 1.

### Essential oil content of *Curcuma aeruginosa* rhizome

The GC-MS method [Table 2] was used to analyze the content of the CAEO. The essential oils in *C. aeruginosa* rhizomes

**Table 1: Percentage yield *Curcuma aeruginosa* rhizome essential oil**

| Number | Area                    | Yield (%) |
|--------|-------------------------|-----------|
| 1      | Kaloran - Temanggung    | 0.108     |
| 2      | Kalibakung - Tegal      | 0.104     |
| 3      | Jumantono - Karanganyar | 0.105     |
| 4      | Bayat - Klaten          | 0.079     |
| 5      | Trucuk - Klaten         | 0.120     |

**Table 2: Gas chromatography-mass spectra chromatography conditions**

| Condition                         | Setting value                      |
|-----------------------------------|------------------------------------|
| GC instrument                     | GC-MS-QP 2010 SE (Shimadzu, Japan) |
| Column/oven temp                  | Agilent® capillary column/75°C     |
| Injection temperature (°C)        | 175                                |
| Flow rate of carrier gas (mL/min) | He at 0.8                          |
| Pressure (kPa)                    | 45.9                               |
| Total flow (mL/min)               | 83.8                               |
| Linier velocity (cm/s)            | 32.9                               |
| Purge flow (mL/min)               | 3.0                                |
| Split ratio                       | 100.0                              |
| Ion source temperature (°C)       | 200                                |
| Interface temperature (°C)        | 225                                |

GC: Gas chromatography, GC-MS: GC-mass spectra

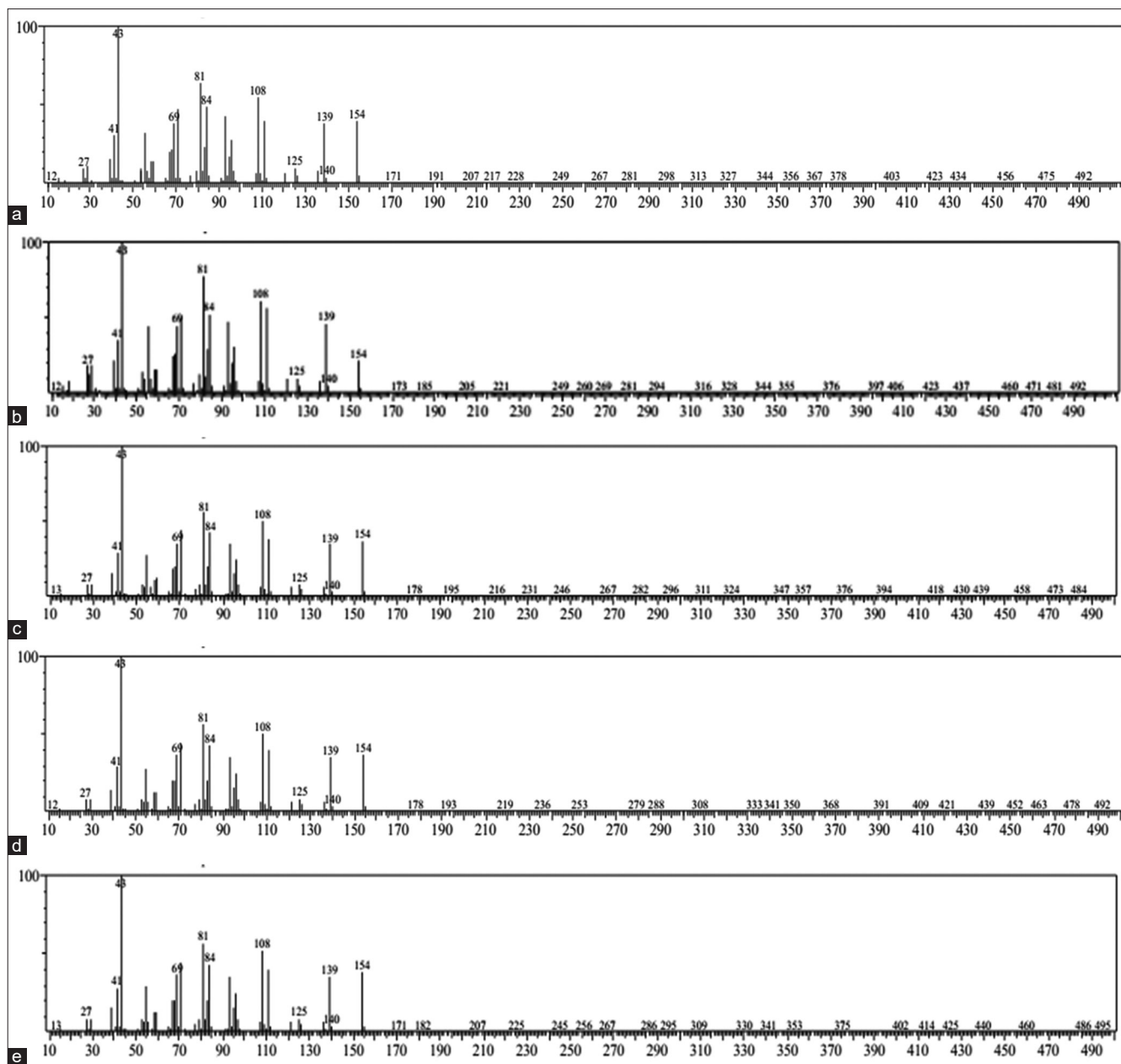


**Figure 1:** Representative chromatogram of *Curcuma aeruginosa* rhizome essential oil from different origins: (a) Kalaran, (b) Kalibakung, (c) Jumantono, (d) Bayat, (e) Trucuk

originating from the five regions contained similar contents but with different levels. Such differences were influenced by the geographical conditions where the plants grew, such as weather, soil height, soil type, soil pH, temperature, and humidity, thus influencing the levels of plant metabolite content.<sup>[19]</sup> The essential oil content of *C. aeruginosa* rhizomes is shown in Table 3. Table 3 displays the 52 peaks that accounted for 98.04%–99.76% of the volatile compounds. However, only 8–12 major compounds were identified, with a more than 2% peak area. The 1,8-cineole compound was found to have the highest level in the essential oils from the five regions. The camphor and isocurcumenol compounds had different essential oil compositions among

the five regions. Variations in the chemical composition of essential oils may be caused by differences in geographical origins and environmental conditions, leading to differences in the quality of *C. aeruginosa* rhizome [Figure 1].

The compound 1,8-cineole is a compound contained in CAEO that is thought to inhibit Wnt/ $\beta$ -catenin activity in HNSCC through decreased GSK-3 inhibition, which leads to decreased levels of WNT11<sup>[20]</sup> and inhibiting the growth of MCF-7 cancer cells,<sup>[21]</sup> likewise as an antibacterial<sup>[22]</sup> and anti-inflammatory.<sup>[23]</sup> Another compound also identified in the CAEO is camphor, capable of inhibiting the cell cycle and inducing apoptosis through the mitochondrial pathway in HCT-116 cells

Table 3: Essential oil content of *Curcuma aeruginosa* rhizomes (2 replications)

| Compound                                                                        | Molecular weight                               | Concentration (%)   |                   |                        |               |                |
|---------------------------------------------------------------------------------|------------------------------------------------|---------------------|-------------------|------------------------|---------------|----------------|
|                                                                                 |                                                | Kaloran, Temanggung | Kalibakung, Tegal | Jumantono, Karanganyar | Bayat, Klaten | Trucuk, Klaten |
| Alpha-pinene                                                                    | C <sub>10</sub> H <sub>16</sub>                | 0.35                | 0.24              | 0.55                   | 0.53          | 0.65           |
| Camphene                                                                        | C <sub>10</sub> H <sub>16</sub>                | 0.93                | 0.73              | 1.21                   | -             | 1.58           |
| Beta-pinene                                                                     | C <sub>10</sub> H <sub>16</sub>                | 1.28                | -                 | 1.64                   | 1.52          | 1.52           |
| Beta-myrcene                                                                    | C <sub>10</sub> H <sub>17</sub>                | -                   | -                 | 0.21                   | 0.16          | 0.19           |
| 1.8-cineole                                                                     | C <sub>10</sub> H <sub>18</sub> O              | 26.66               | 28.38             | 34.01                  | 29.35         | 30.93          |
| Camphor                                                                         | C <sub>10</sub> H <sub>16</sub> O              | 11.47               | 19.05             | 3.24                   | 18.37         | 18.86          |
| 2-undecanol                                                                     | C <sub>11</sub> H <sub>24</sub> O              | -                   | 0.47              | 0.46                   | -             | -              |
| Endo-borneol                                                                    | C <sub>10</sub> H <sub>18</sub> O              | 0.70                | 1.09              | 0.91                   | 0.92          | 1.00           |
| 4-terpineol                                                                     | C <sub>10</sub> H <sub>18</sub> O              | 0.46                | 1.00              | 0.89                   | 0.82          | 0.90           |
| Alpha-terpinyl acetate                                                          | C <sub>12</sub> H <sub>20</sub> O <sub>2</sub> | 0.74                | 1.75              | 0.16                   | 1.41          | 1.50           |
| Beta-elements                                                                   | C <sub>15</sub> H <sub>24</sub>                | 0.56                | 0.49              | 0.48                   | 0.85          | 0.58           |
| Trans-caryophyllene                                                             | C <sub>15</sub> H <sub>24</sub>                | 0.24                | 0.18              | 0.48                   | 0.20          | 0.18           |
| Beta-farnesene                                                                  | C <sub>15</sub> H <sub>24</sub>                | -                   | -                 | 0.28                   | 0.27          | 0.20           |
| Alpha-humulene                                                                  | C <sub>10</sub> H <sub>16</sub>                | 1.83                | -                 | -                      | 0.21          | -              |
| Germacrene D                                                                    | C <sub>15</sub> H <sub>24</sub>                | 0.22                | 0.18              | 0.21                   | 0.19          | 0.26           |
| Beta-selinene                                                                   | C <sub>15</sub> H <sub>24</sub>                | -                   | 0.15              | -                      | 0.14          | 0.17           |
| Curzerene                                                                       | C <sub>15</sub> H <sub>20</sub> O              | 0.43                | 0.41              | 0.35                   | 0.41          | 0.41           |
| Germacrene A                                                                    | C <sub>15</sub> H <sub>24</sub>                | 0.17                | 0.15              | 0.16                   | 0.15          | 0.19           |
| Germacrene B                                                                    | C <sub>15</sub> H <sub>24</sub>                | 0.17                | -                 | 0.15                   | -             | 0.18           |
| Epiglobulol                                                                     | C <sub>15</sub> H <sub>26</sub> O              | 0.40                | 0.46              | 0.40                   | 0.41          | 0.39           |
| Elemol                                                                          | C <sub>15</sub> H <sub>26</sub> O              | 0.29                | 0.26              | 0.22                   | 0.24          | 0.30           |
| Boldenone                                                                       | C <sub>19</sub> H <sub>26</sub> O <sub>2</sub> | 19.06               | -                 | 14.97                  | 14.53         | 13.90          |
| Isocurcumenol                                                                   | C <sub>15</sub> H <sub>22</sub> O <sub>2</sub> | 2.86                | 8.89              | 5.97                   | 5.55          | 4.68           |
| Juniper camphor                                                                 | C <sub>15</sub> H <sub>26</sub> O              | -                   | -                 | 1.47                   | 1.58          | 1.94           |
| 1 (10), 4-aromedenradiene                                                       | C <sub>15</sub> H <sub>22</sub>                | -                   | -                 | 0.51                   | 0.55          | 0.54           |
| Spathulenol                                                                     | C <sub>15</sub> H <sub>24</sub> O              | -                   | -                 | 1.08                   | 1.44          | 0.15           |
| Germacrone                                                                      | C <sub>15</sub> H <sub>22</sub> O              | -                   | 3.38              | 3.14                   | 3.62          | 3.45           |
| Aromadendrene epoxide                                                           | C <sub>15</sub> H <sub>24</sub> O              | -                   | -                 | 1.84                   | 0.48          | 0.50           |
| Trans-longipinocarveol                                                          | C <sub>15</sub> H <sub>24</sub> O              | -                   | 0.41              | 0.57                   | -             | -              |
| Germacrol                                                                       | C <sub>15</sub> H <sub>22</sub> O              | -                   | -                 | 3.42                   | -             | -              |
| 3-methyl-5-(2,6,6-trimethyl-1-cyclohexene-1-yl)-1-pentyl-3-ol                   | C <sub>15</sub> H <sub>24</sub> O              | 1.44                | 1.73              | -                      | -             | 0.45           |
| (E)-2,3,3-Trimethyl-2-(3'-methyl-1'-3'-butadiene-1'-yl)-6-methyldicyclohexanone | C <sub>26</sub> H <sub>38</sub> O              | 3.27                | 2.97              | -                      | -             | 2.48           |
| Tricyclo[5.1.0.0 (3.5)]octane-2,6-dione. 1.3.5.7-tetramethyl-                   | C <sub>16</sub> H <sub>28</sub> O <sub>2</sub> | -                   | -                 | 3.69                   | 3.49          | -              |
| Cyclohexane. 1.1-dimethyl-2.4-bis (1-methyl ethenyl)-, cis-                     | C <sub>15</sub> H <sub>26</sub>                | 5.16                | -                 | 3.84                   | -             | -              |
| 2-cyclohexen-1-one, 4-ethynyl-4-hydroxy-3,5,5-trimethyl-                        | C <sub>10</sub> H <sub>14</sub> O              | -                   | 15.95             | -                      | -             | -              |
| 3,7-cyclodecadien-1-one, 10-(1-methyl ethenyl)-, (E,E)                          | C <sub>15</sub> H <sub>22</sub> O              | 3.15                | -                 | -                      | -             | -              |

Contd...

Table 3: Contd...

| Compound                                                                         | Molecular weight                               | Concentration (%)   |                   |                        |               |                |
|----------------------------------------------------------------------------------|------------------------------------------------|---------------------|-------------------|------------------------|---------------|----------------|
|                                                                                  |                                                | Kaloran, Temanggung | Kalibakung, Tegal | Jumantono, Karanganyar | Bayat, Klaten | Trucuk, Klaten |
| Dihydrocarvone                                                                   | C <sub>10</sub> H <sub>16</sub> O              | -                   | 4.01              | -                      | 4.47          | 4.33           |
| Calarene                                                                         | C <sub>15</sub> H <sub>24</sub>                | 5.39                | -                 | 3.33                   | 3.93          | 4.12           |
| Bicyclo[2.2.2]Octa-2.5-Diene. 1.2.3.6-tetramethyl                                | C <sub>12</sub> H <sub>18</sub>                | -                   | 4.70              | -                      | -             | -              |
| Ledenoxide-(II)                                                                  | C <sub>15</sub> H <sub>24</sub> O              | 0.96                | 0.86              | 0.72                   | 1.11          | 0.86           |
| Curdione                                                                         | C <sub>15</sub> H <sub>24</sub> O <sub>2</sub> | 2.76                | -                 | -                      | -             | -              |
| Ledane                                                                           | C <sub>15</sub> H <sub>26</sub>                | -                   | -                 | 2.84                   | -             | -              |
| 1H-cyclopropa(a)naphthalene, 1A,2,4,5,6,7,7A,7B-octahydro-1,1,7,7A-              | C <sub>15</sub> H <sub>24</sub>                | -                   | -                 | 3.59                   | -             | -              |
| (E)-2,3,3-trimethyl-2-(3'-methyl-1'-3'-butadien-1'-yl)-6-methylidencyclohexanone | C <sub>14</sub> H <sub>22</sub> O              | -                   | -                 | 2.32                   | 2.75          | -              |
| Tricyclo[5.2.2.0 (1.6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-                | C <sub>15</sub> H <sub>24</sub> O              | 0.66                | -                 | -                      | -             | -              |
| (3E)-5-isopropyliden-6-methyl-3,6,9-decatrien-2-one                              | C <sub>14</sub> H <sub>20</sub> O              | 5.04                | -                 | -                      | -             | -              |
| Trans-p-mentha-1 (7),8-dien-2-ol                                                 | C <sub>10</sub> H <sub>16</sub> O              | 0.89                | -                 | -                      | -             | -              |
| Hexadecane, 2,6,10,14-tetramethyl-                                               | C <sub>20</sub> H <sub>42</sub>                | 0.42                | -                 | -                      | -             | -              |
| Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-7-octyl-                            | C <sub>20</sub> H <sub>32</sub>                | 0.60                | -                 | -                      | -             | -              |
| Tetradecane, 1-chloro-                                                           | C <sub>14</sub> H <sub>29</sub> Cl             | 0.90                | -                 | -                      | -             | -              |
| Phenanthrene. 1.2.3.4.5.6.7.8-Octahydro-                                         | C <sub>15</sub> H <sub>17</sub> NO             | -                   | 0.22              | 0.17                   | 0.15          | 0.69           |
| Total identified (%)                                                             |                                                | 99.46               | 98.11             | 99.45                  | 99.76         | 98.04          |
| Monoterpene hydrocarbons (%)                                                     |                                                | 4.39                | 0.97              | 3.61                   | 2.42          | 3.93           |
| Oxygenated monoterpenes (%)                                                      |                                                | 40.92               | 71.70             | 39.66                  | 55.32         | 57.51          |
| Sesquiterpene hydrocarbons (%)                                                   |                                                | 11.91               | 1.15              | 18.19                  | 6.28          | 6.41           |
| Oxygenated sesquiterpenes (%)                                                    |                                                | 37.05               | 16.40             | 34.14                  | 32.10         | 27.15          |
| Oxygenated diterpenes (%)                                                        |                                                | 1.02                | -                 | -                      | -             | -              |
| Others (%)                                                                       |                                                | 4.17                | 7.89              | 3.86                   | 3.64          | 3.17           |

*in vitro* by 30% and then stopping cell proliferation.<sup>[24]</sup> The use of camphor relieves pain, itchiness, and irritation in the affected area by activating particular transient receptor potential (TRP) channels, such as TRPV1, TRPV3, and TRPM8, and by inhibiting TRPA1. Camphor also produces a warm sensation and desensitizes sensory nerve endings.<sup>[25]</sup> Many pharmacological uses for camphor include expectorant, cough

suppressant, rubefacient, antiseptic, antipruritic, anti-infective, anti-spasmodic, nasal decongestant, and cough suppression.<sup>[26]</sup>

### Fourier Transform Infrared Spectroscopy analysis

Data for each *C. aeruginosa* rhizome essential oil sample were obtained by scanning the results with an FTIR using ATR. Scanning was performed in five replications

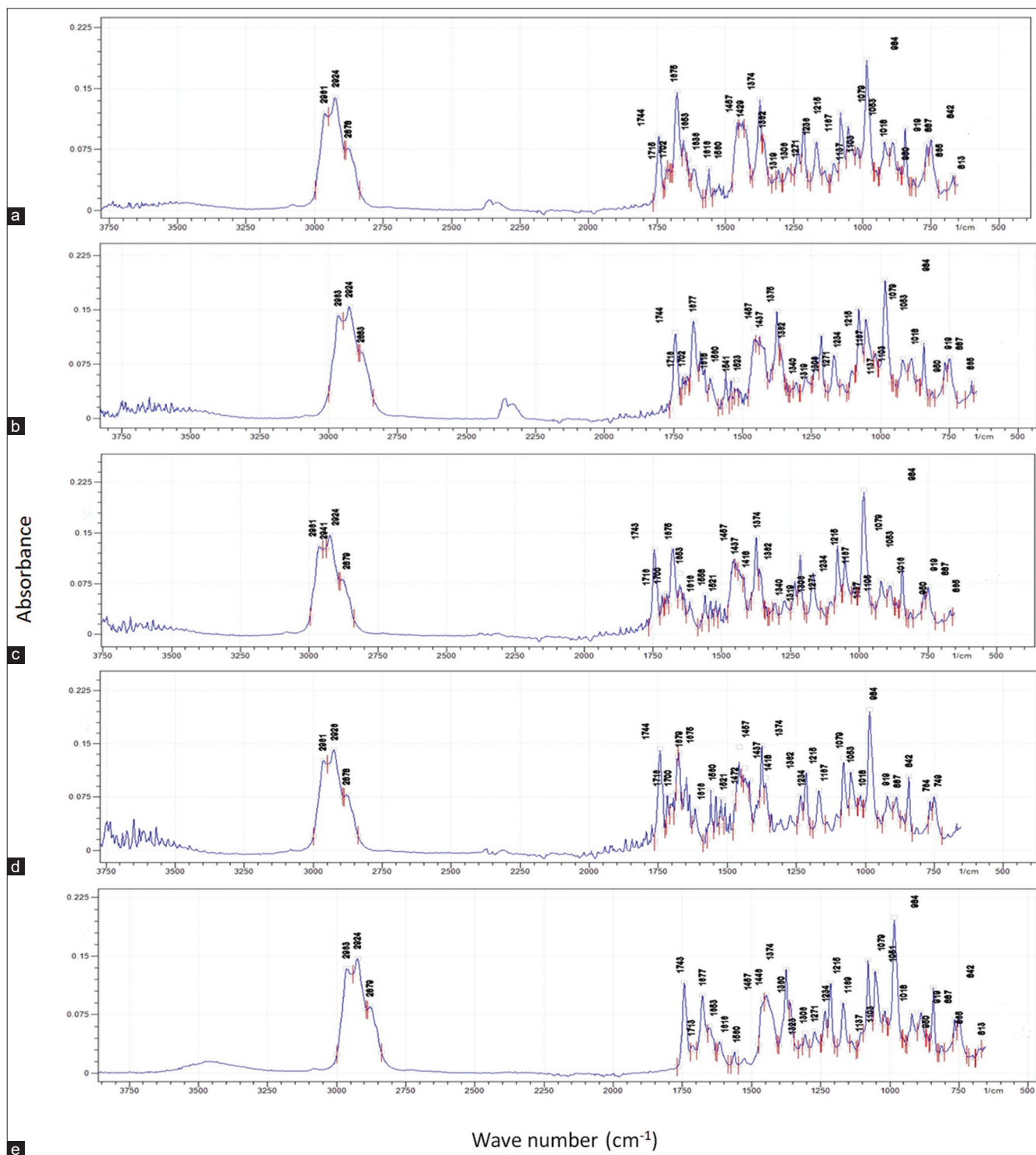


**Figure 2:** *Curcuma aeruginosa* rhizome essential oil from different regions: (a) Caloran, (b) Kalibakung, (c) Jumantono, (d) Bayat, (e) Trucuk

in waves ranging from 4000 to 650 per cm. Wave number 1750–700 per cm was a fingerprint area with a resolution of 8/cm.

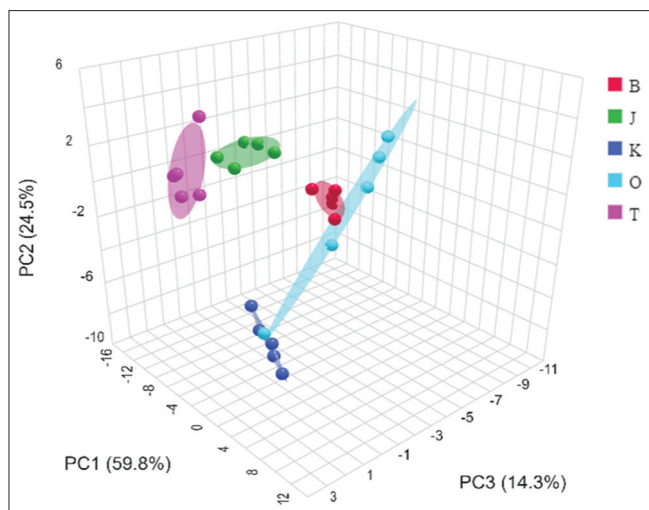


**Figure 3:** Results principal component analysis

The infrared spectrum of CAEO was taken from five geographically different regions [Figure 2], namely Kaloran, Kalibakung, Jumanthoro, Bayat, and Trucuk. The fingerprint area at wave numbers 1750–700 per cm showed a similar spectrum pattern, but there were differences in each absorption value due to the content or concentration of compounds in each CAEO. The CAEO had varying absorption bands: band 1 (3000–2750 per cm) showing the CH group (methyl or methylene) in the aliphatic chain in the essential oil; band 2 (around 1725–1675 per cm) showing the C = O (ketone) group; band 3 (1650–1475 per cm) showing C = C (alkene); and band 4 (1300–1000 per cm), indicating the presence of stretching vibrations of CO in compounds containing ether or alcohol. These bands [Figure 2] showed that they contained the exact contents of chemical compounds but with different levels.

The PCA method was applied using software *metaboanalysis* 6.0 which is freely available at <https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml>, resulting in a number of data in the form of score plots and biplots.

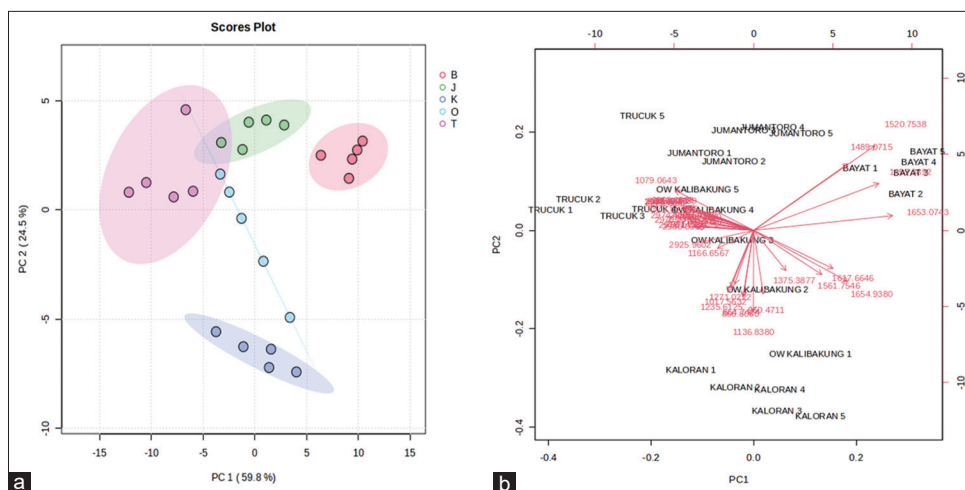


**Figure 4:** Principal component analysis results: (a) score plot; (b) biplot on Fourier Transform Infrared Spectroscopy

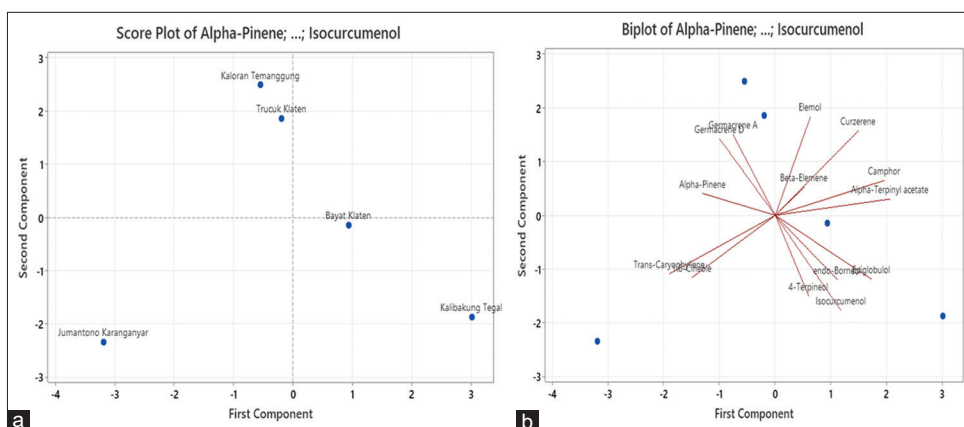


**Figure 5:** Principal component analysis results: (a) score plot; (b) biplot on gas chromatography-mass spectra

A score plot was used to present a plot of the relationship or similarity between the CAEO from the five regions based on the principal component (PC) values. In this study, two PCs were used, namely PC1 and PC2, marked with points with a certain distance between one point and another. Regarding PC, objects with almost the same or close PC values can be said to have similar physicochemical properties. Therefore, PCA can be used for object grouping purposes.<sup>[27]</sup>

A biplot is a combination of a score plot and a loading plot. The loading plot shows how strongly each variable influences the PC by depicting it as a vector. If two vectors form an angle of  $<90^\circ$ , the two variables correlate positively. If it forms a right angle or around  $90^\circ$ , the two variables cannot correlate. The two variables show a negative correlation if they form an angle  $>90^\circ$ – $180^\circ$ .<sup>[28]</sup>

### Principal component analysis of *Curcuma aeruginosa* essential oil

In Figure 3, the highest eigenvalue results were shown in PC1 and then PC2, which means that these values

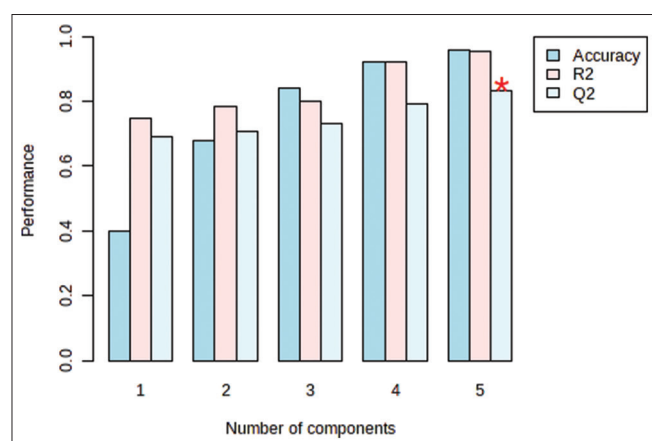


**Figure 6:**  $R^2$  and  $Q^2$  intercept values from permutations in partial least squares discriminant analysis on Fourier Transform Infrared Spectroscopy. “\*” indicates that the model has excellent predictive performance, with  $Q^2 \geq 0.7$

contributed to the compound content of 59.8% on PC1 and 24.5% on PC2. In this analysis, PC1 and PC2 can be used to represent 100% of the variables (cumulative).

The relationship between the five essential oils of *C. aeruginosa* was different, as seen by the score plot and biplot displayed in Figure 4. The score plot displays five points of essential oils from the five locations distant from one another. The conditions of the soil, temperature, and weather at the location of *C. aeruginosa* rhizomes grow are the causes of the dissimilarity. Because of this, the five rhizomes differed in terms of the number of compounds contained, depending on the location in which they grew. The PCA results displayed in Figure 5 demonstrated a favorable association between curzerene and beta element compounds' antibacterial and anti-inflammatory properties when compared to elemol and germacrene. A compound is considered to include essential oil components if its angle is smaller than  $90^\circ$ . The rhizome of *C. aeruginosa*, specifically beta-element, curzerene, elemol, and germacrene D, has antibacterial and antioxidant properties. Curzerene<sup>[29]</sup> and germacrene<sup>[30]</sup> have been studied for their antioxidant potential. According to Sieniawska *et al.* (2018), the beta-element in *Nigella damascena* has moderate to strong antibacterial activity against *Mycobacterium tuberculosis*.<sup>[31]</sup> The essential oil of *Neomirandea angularis* leaves contains *Bacillus cereus*, which is the target of germacrene D's antibacterial activity.<sup>[32]</sup>

### Partial least squares discriminant analysis

To obtain a high level of group separation and improve variable recognition for sample classification, the PLS-DA technique was used. 3D PLS-DA obtained on the sample distribution [Figure 6] based on the wave numbers identified in the CAEO showed that all the test samples could be clearly classified into five groups according to their origin.

The main purpose of permutations in PLS-DA is to test the significance of the model that has been built and evaluate

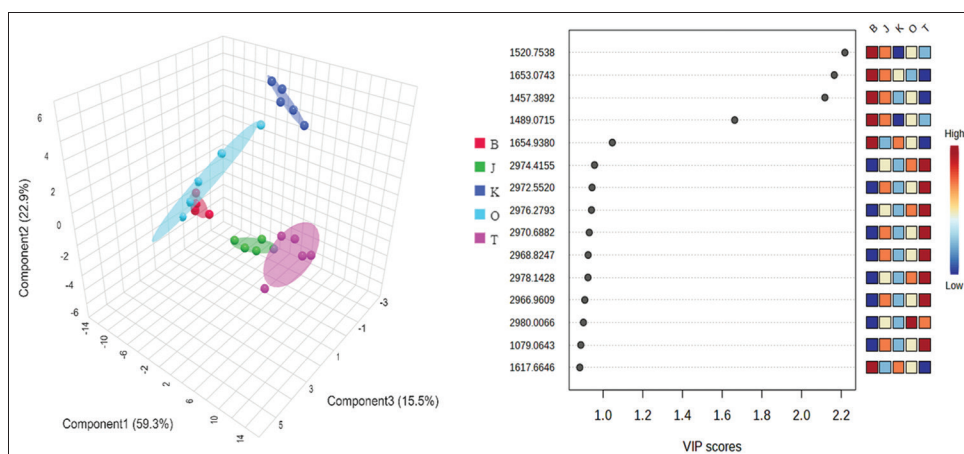


**Figure 7:** Partial least squares discriminant analysis results and VIP values on Fourier Transform Infrared Spectroscopy

the model.  $R$ -squared ( $R^2$ ) and  $Q$ -squared ( $Q^2$ ) are evaluation parameters often used in the context of discriminant PCA with the PLS-DA method. However, the  $R^2$  and  $Q^2$  values are not obtained directly from permutations but are often used in internal cross-validation of PLS-DA models.  $R^2$  on PC2 was 0.79, where the higher the value, the better the model at explaining variations in response data, while  $Q^2$  on PC2 was 0.70, which was obtained from evaluation through cross-validation; the higher the value, the better the model to predict new data. Some studies use specific significance levels (e.g.,  $\alpha=0.05$ ) to determine whether  $R^2$  and  $Q^2$  values are significant. If the original values are higher or lower than the values resulting from permutation at the selected significance level, the model is considered significant. The above results indicated that the PLS-DA technique would be more suitable than PCA to provide visual differentiation of test samples according to origins regarding quality control.

Based on PLS-DA, the loading plot shows the contribution of each to the sample discrimination variable. Based on Variable Importance Projection (VIP) values in Figure 7, some wave numbers had VIP values  $>1.5$ , so it can be concluded that wave numbers  $g$  of 1489.0715; 1475.3892; 1520.7538; and 1653.0743 per cm were the most important wave numbers in showing differences in *C. aeruginosa* rhizome essential oil.

## CONCLUSION

*C. aeruginosa* rhizome is a traditional herb widely used in many countries. This study proposed an efficient strategy for quality control of CAEO as raw materials in the pharmaceutical and natural products industry. Even though there were variations in the chemical contents due to differences in geographical origins, the GC-MS fingerprint profiles of the essential oil samples were similar based on visual inspection, making it difficult to classify the essential oil samples according to their different origins. Therefore, the chemometric technique was applied with the FTIR fingerprint technique to recognize CAEO from various origins. It has shown that some techniques, such as PCA and PLS-DA, could classify the samples objectively. Furthermore, four-wave numbers were the most important variables in differentiating *C. aeruginosa* rhizomes for the quality control of CAEO using quantitative analysis.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Sari AP. Phytochemistry and biological activities of *Curcuma aeruginosa* (Roxb.). *Indones J Chem* 2022;22:576-98.
2. Kamazeri TS, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. *Asian Pac J Trop Med* 2012;5:202-9.
3. George M, Britto SJ. Phytochemical and antioxidant studies on the essential oil of The Rhizome of *Curcuma aeruginosa* Roxb. *Int Res J Pharm* 2015;6:573-9.
4. Theanphong O, Mingvanish W, Kirdmanee C. Chemical constituents and biological activities of essential oil from *Curcuma aeruginosa* Roxb. Rhizome. *Sci Technol BHST* 2015;13:6-16. Doi: <https://li05.tci-thaijo.org/index.php/IJHS/article/view/105>.
5. Batubara I, Yunita D, Suparto IH. Antibacterial and biofilm degradation activity of extract from steam distillation residue of *Zingiberaceae* leaves against *Streptococcus mutans*. *J Indones Chem Soc* 2019;2:42.
6. Mohammadhosseini M, Akbarzadeh A, Hashemi-Moghaddam H, Bahmanpour H, Shafaghath A, Lotfi S. The relationship between chemical composition of the essential oils of *Platycladus orientalis* (L.) Franco and soils contamination in national oil company of Shahrood, Iran. *J Essent Oil Bearing Plants* 2017;20:1209-25.
7. Dosoky NS, Satyal P, Setzer WN. Variations in the volatile compositions of *Curcuma* species. *Foods* 2019;8:1-14. doi:10.3390/foods8020053.
8. Poudel DK, Ojha PK, Rokaya A, Satyal R, Satyal P, Setzer WN. Analysis of volatile constituents in curcuma species, viz. *C. aeruginosa*, *C. zedoaria*, and *C. longa*, from Nepal. *Plants (Basel)* 2022;11:1932.
9. Popa RM, Fetea F, Socaciu C. ATR-FTIR-MIR spectrometry and pattern recognition of bioactive volatiles in oily versus microencapsulated food supplements: Authenticity, Quality, and Stability. *Molecules* 2021;26:4837.
10. Truzzi E, Marchetti L, Bertelli D, Benvenuti S. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy coupled with chemometric analysis for detection and quantification of adulteration in lavender and citronella essential oils. *Phytochem Anal* 2021;32:907-20.
11. Chun MH, Kim EK, Yu SM, Oh MS, Moon KY, Jung JH, et al. GC/MS combined with chemometrics methods for quality control of *Schizonepeta tenuifolia* Briq: Determination of essential oils. *Microchem J* 2011;97:274-81.
12. Ahmad I, Fikri JA, Arifianti AE, Abdullah S, Mun'im A. The combination of ATR FTIR and chemometrics for rapid analysis of essential oil from Myrtaceae plants – A review. *J Appl Pharm Sci* 2022;12:30-42.
13. Syafri S, Jaswir I, Yusof F, Rohman A, Ahda M, Hamidi D. The use of instrumental technique and chemometrics for essential oil authentication: A review. *Results Chem* 2022;4:1-10. [doi: 10.1016/j.rechem. 2022.100622].
14. Ruiz-Perez D, Guan H, Madhivanan P, Mathee K, Narasimhan G. So you think you can PLS-DA? *BMC Bioinformatics* 2020;21:2.

15. Angel G. Isolation and evaluation of bioactive non starch polysaccharides from eight starchy *Curcuma* species. *Nat Prod J* 2013;3:204-11.
16. Hirri A, Bassbasi M, Platikanov S, Tauler R, Oussama A. FTIR spectroscopy and PLS-DA classification and prediction of four commercial grade virgin olive oils from morocco. *Food Methods* 2016;9:974-81.
17. Agatonovic-Kustrin S, Ristivojevic P, Gegechkori V, Litvinova TM, Morton DW. Essential oil quality and purity evaluation via ft-ir spectroscopy and pattern recognition techniques. *Appl Sci* 2020;10:1-12.
18. Ministry of Health of the Republic of Indonesia, "Indonesian Herbal Pharmacopoeia 2<sup>nd</sup> Edition, Ministry of Health of the Republic of Indonesia," Indonesia. 2017.
19. Dosoky NS, Setzer WN. Chemical composition and biological activities of essential oils of curcuma species. *Nutrients* 2018;10:1196.
20. Roettger A, Bruchhage KL, Drenckhan M, Ploetze-Martin K, Pries R, Wollenberg B. Inhibitory effect of 1,8-cineol on  $\beta$ -catenin regulation, WNT11 expression, and cellular progression in HNSCC. *Front Oncol* 2017;7:92.
21. Fitria R, Seno DS, Priosoeryanto BP, Hartanti, Nurcholis W. Volatile compound profiles and cytotoxicity in essential oils from the rhizome of *Curcuma aeruginosa* and *Curcuma zanthorrhiza*. *Biodiversitas* 2019;20:2943-8.
22. Şimşek M, Duman R. Investigation of effect of 1,8-cineole on antimicrobial activity of chlorhexidine gluconate. *Pharmacognosy Res* 2017;9:234-7.
23. Jiang Z, Luo M, Ma W, Ma S, Wang Y, Zhang K. Protective effects of 1,8-cineole microcapsules against inflammation and gut microbiota imbalance associated weight loss induced by heat stress in broiler chicken. *Front Pharmacol* 2020;11:585945.
24. Itani WS, El-Banna SH, Hassan SB, Larsson RL, Bazarbachi A, Gali-Muhtasib HU. Anti colon cancer components from Lebanese sage (*Salvia libanotica*) essential oil: Mechanistic basis. *Cancer Biol Ther* 2008;7:1765-73.
25. Singh R, Jawaid T. *Cinnamomum camphora* (Kapur): Review. *Pharmacogn J*. 2012;4:1-5.
26. Hamidpour R, Hamidpour S, Hamidpour M, Hamidpour R. The Effect of Camphor Discovery for Treating Asthma Corresponding Author. Vol 2. Available from: <https://www.opastonline.com>.
27. Riyanta AB, Riyanto S, Lukitaningsih E, Rohman A. The employment of fourier transform infrared spectroscopy (FTIR) and chemometrics for analysis of candlenut oil in binary mixture with grape seed oil. *Food Res* 2020;4:184-90.
28. Widodo H, Sisindari S, Asmara W, Rohman A. Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *J Appl Pharm Sci* 2019;9:99-105.
29. da Costa JS, Barroso AS, Mourão RH, da Silva JK, Maia JG, Figueiredo PL. Seasonal and antioxidant evaluation of essential oil from *Eugenia uniflora* L., curzerene-rich, thermally produced *in situ*. *Biomolecules* 2020;10:328.
30. Casiglia S, Bruno M, Bramucci M, Quassinti L, Lupidi G, Fiorini D, *et al.* *Kundmannia sicula* (L.) DC: A rich source of germacrene D. *J Essent Oil Res* 2017;29:437-42.
31. Sieniawska E, Sawicki R, Golus J, Swatko-Ossor M, Ginalska G, Skalicka-Wozniak K. *Nigella damascena* L. essential oil-a valuable source of  $\beta$ -elemene for antimicrobial testing. *Molecules* 2018;23.
32. Washington VD, Palazzo MC, Haber WA, Setzer WN. The chemical composition and antibacterial activity of the leaf essential oil of *Neomirandea angularis* (Asteraceae) from monteverde, Costa Rica. *J Essent Oil-Bearing Plants* 2010;13:108-11.