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Original Research Article

Influence of Geographical Origin on Essential Oil Contents in *Curcuma aeruginosa* Roxb. Rhizomes in Central Java, Indonesia

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ABSTRACT

Curcuma aeruginosa Roxb. rhizome is a traditional herb used in several countries. Depending on where they are from, *Curcuma aeruginosa* Roxb. rhizomes can have different amounts of compounds. The purpose of this study was to evaluate the influence of geographical origin on the composition of essential oils extracted from the rhizomes of *Curcuma aeruginosa* Rox throughout the distribution area in Central Java, Indonesia using Gas Chromatography-Mass Spectrometry (GC-MS). The chemometrics of Principal Component Analysis (PCA) and Partial Least Squares Discriminative Analysis (PLS-DA) have been successfully employed for the classification of samples into different classes. The sand, clay, organic carbon, and yield were variables contributing significantly to PLS-DA modelling during the relationship among the sample locations, environmental factors, and micronutrient contents with the variable importance projection (VIP) score of >1. Curzerenone is considered the most important variable contributing to PLS-DA modelling for the relationship between the chemical compounds of *C. aeruginosa* essential oils (CA-EOs) with the sample locations and soil micronutrients. This study showed that PCA and PLS-DA can offer a reliable platform for grouping and classifying samples, therefore, the determination of the variables contributing significantly toward the geographical origins of samples is essential since these variables affected the contents of CA-EOs. In addition, this research can help researchers and farmers choose the most suitable soil types to achieve the maximum yields of CA-EOs.

Keywords: Gas chromatography-mass spectrometry, environmental factors, edaphic, *C. aeruginosa*, chemometrics.

Introduction

Central Java Indonesia encompasses approximately 32.800 km^2, constituting 25.04% of Java Island. Indonesia has produced the medicinal plants commonly used in traditional medicines such as *Kaempferia galanga, Curcuma longa, Curcuma xanthorrhiza, Curcuma zedoaria, Alpinia galanga, Zingiber officinale*, and *Curcuma aeruginosa*.[1] These medicinal plants are widely found in Central Java and grow well in both lowlands and highlands, therefore, Central Java has become one of the largest traditional medicine business centres in Indonesia.[2] The most of *C. aeruginosa* commonly used in traditional medicine is the rhizome.[3] The essential oils of *C. aeruginosa* (CA-EOs) have been reported to have some biological activities having beneficial effects on human health including antioxidants,[4] antimicrobial,[5] anticancer,[6] and anti-androgenic.[7] Therefore, many studies have been developed to increase the production of essential oils (EOs) in medicinal plants.

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Several studies have been reported to examine the influence of geographic origins and environmental conditions on the types and contents of EOs. Marčetic' *et al*.[8] reported that there was no significant effect of climate and soil types on the yield of EOs of *Seseli rigidum* Waldst. & Kit roots from the different locations in Serbia. Rahimmalek *et al*.[9] studied the variation of EOs in Iranian Ajowan (*Trachyspermum ammi* (L.) Sprague collected from the different geographical regions with climatic factors and reported that high contents of EOs and thymol were found in relatively rocky soils and dry conditions. Aboukhalik *et al*.[10] studied the effect of environmental factors on the variability of EOs of *Origanum compactum* Benth. which grows wild in Morocco, and the results showed a significant relationship between the yield of EOs and several edaphic factors (soil pH, K_2O content, soil texture). High levels of carvacrol content were found in semi-arid climate conditions, growing in highlands, and muddy soils, while high thymol content was associated with humid climates, clay, and sandy soils. These studies confirmed that some physical, chemical, and biological factors can influence the quality and quantity of EO composition. Some environmental factors including weather, altitude, and temperature,[11-12] soil type, pH, and humidity soil[13-14] can contribute to quantitative and qualitative changes in EOs.

The standard method for analysis of EO composition is gas chromatography (GC) using a mass spectrometer (GC-MS) and flame ionization detectors (GC-FID).[15-16] The data generated from the measurements of environmental factors and micronutrient contents in the soil associated with the composition of CA-EOs is very large, therefore, the use of chemometrics or multivariate data analysis is indispensable.[9-10] The chemometrics approaches based on pattern recognition techniques such as Principal Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA) are

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commonly used for the classification of medicinal plants using some variables of environmental conditions, macronutrient contents in soil, and phytochemical compositions. This study aimed to evaluate the influence of geographical origin on the composition of essential oils extracted from the rhizomes of *Curcuma aeruginosa* Roxb. throughout the distribution area in Central Java, Indonesia using Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods

Plant collection and identification

The samples used in this research were rhizomes of *C. aeruginosa* from ten regions with three replications, harvested in July-August 2023. Thirty *C. aeruginosa* samples were obtained from city and district areas in Central Java, Indonesia (Figure 1). The authenticity of *C. aeruginosa* samples was determined by the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia with authentication letter Number 19.29.11/UN1/FFA.2/S1/PT/2022.

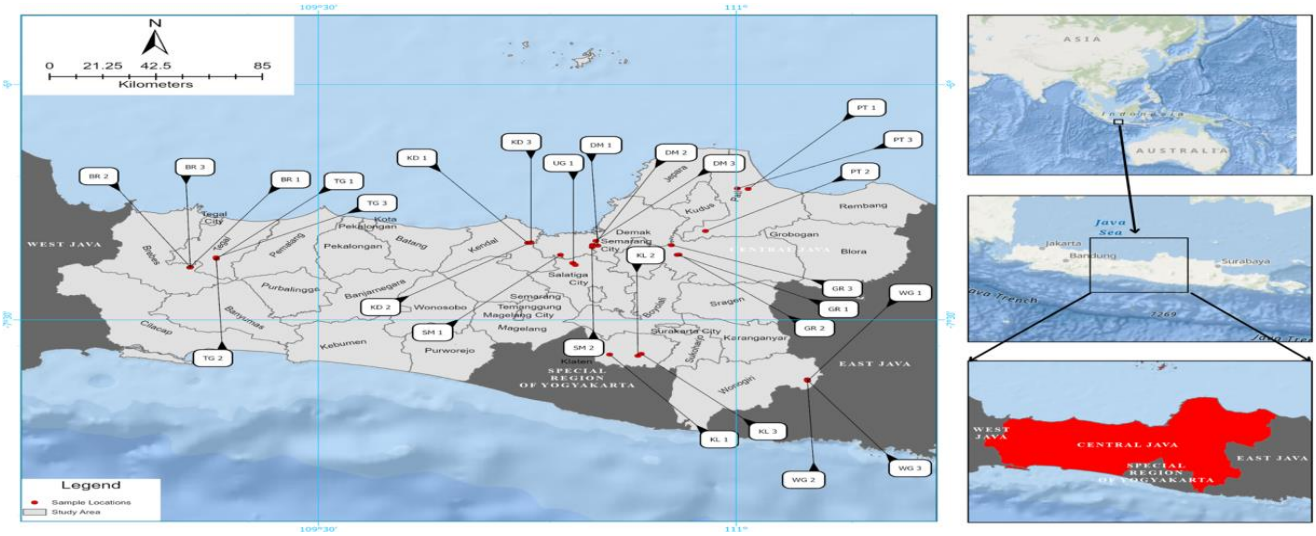

Figure 1: Geographical map of sampling location for *Curcuma aeruginosa* (source: QGIS)

Preparation of essential oils

Fresh rhizomes of *C. aeruginosa* were sliced with a thickness of approximately 1-2 cm and then steamed and distilled for 8 hours over medium heat.[17] The resulting distillate was separated from water by adding anhydrous sodium sulphate and subjected to centrifugation at 2500 rpm (Thermo Scientific, Massachusetts Amerika) for 5 min.

Analysis of essential oil profiles using GC-MS

Compositional analysis of CA-EOs was carried out using GC-MS (GC-MS-QP2010 SE Shimadzu, Japan). The data was obtained from gas chromatography (GC) and mass spectrometry (MS). The GC instrument (GC-MS-QP2010 SE (Shimadzu, Japan), used the following conditions: Column/oven Temp: Rtx 5 MS column/50°C, Injection Temperature of 300°C at a flow rate of carrier gas (He) of 0.66 mL/min, column pressure: 24.7 kPa and total flow: 90.6 mL/min and linear velocity of 29.5 cm/sec, purge flow of .9 mL/min, split ratio: 129.9. The ion Source Temp of 250°C, interface temp. 300°C, mass acquisition: start m/z: 28.00 and end m/z: 600.00.

Determination of Soil Texture

A kilogram of soil sample with a size of <2 mm and 50 mL of 10% H_2O_2 solution was mixed and left for 24 hours. After that, 25 mL of 30% H_2O_2 was added and heated until there were no foams. This was followed with 180 mL of ion-free water and 20 mL of 2N HCl, and the mixture was heated for 10 minutes. After cooling, the solution was diluted with 700 mL of aqua demineralisation, filtered until it was acid-free, and then 0 mL of 4% $Na_2P_2O_7$ peptization solution was added. After that, the sand, silt, and clay were separated.[18]

Soil Analysis

The soil around *C. aeruginosa* growth was tested for the soil's moisture and pH value using a Soil Moisture meter pH tester (2 in 1 VT05, Indonesia). The soil samples were taken at a depth of 0-20 mm; then, the contents of micronutrients in the soil were also carried out, i.e. organic carbon (OC), nitrogen (N), phosphate (P), and potassium (K). The determination was as follows: nitrogen (N) was carried out using the Kjeldahl method (UDK129 Kjedahl Distillation Unit 230V/50-60Hz, Velp Scientifica-Italy),[18] organic carbon (OC) using the digestion method by visible spectrophotometer at λ 561 nm,[18] phosphorus (P) using visible Spectrophotometer 721 (Yoke-China) at λ 889 nm and blue molybdate reagent, while the potassium (K) using Atomic Absorption Spectrophotometer (type AA-7000, Shimadzu-Japan).[19]

Statistical analysis

PCA and PLS-DA were used to classify the environmental and edaphic factors including pH, soil moisture, and soil texture; yield and soil micronutrient contents; as well as the relationship between chemical compounds of CA-EOs and soil micronutrient contents. Analyses of PCA and PLS-DA were carried out using the software Metaboanalyst 6.0, which is freely available at https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml.

Results and Discussion

In this study, the profiles of CA-EOs from various regions in Central Java, Indonesia were determined. The soil samples were taken from the surface layer at a depth of 0-20 cm because, at this depth, there are many nutrients and biological activities in the soil. The selection of samples at 30 different growing locations was carried out based on the availability of adequate *C. aeruginosa* plant samples representing the condition of the plant population. The soil samples taken at each location were subjected to physical and chemical characterisations by determining the soil textures (sand, clay, and silt) and soil chemistry (OC, N, P, and K). The chemometrics of PCA were used to evaluate the variables contributing to the classification of sample locations where *C. aeruginosa* grows. Figure 2 shows the scree plot and bi-plot (score plot versus loading plot) of sample locations as characterised by environmental and edaphic factors using two principal components (PC), namely the first PC (PC1) and the second (PC2). PC1 and PC2 contributed to 51.3% and 41.3% variances, respectively; therefore, using two PC2, 92.6% data variances of variables could be described. Sample locations having high clay content and lower soil moisture are

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BR1, BR2, PT3, UG3, and SM1 (Figure 2b). It is influenced by the time of soil sampling, where in July 2023, there is a long dry climate resulting in low rainfall and low drainage at the planting site. Idowu[20] stated that high sand content has a negative influence on soil aggregate stability, while Fernández-Ugalde *et al*.[21] reported that soil content having a large percentage of clay has a positive influence on the stability of soil aggregates. Sand tends to have low aggregate stability due to its large size and low surface area compared to clay having a high surface area.

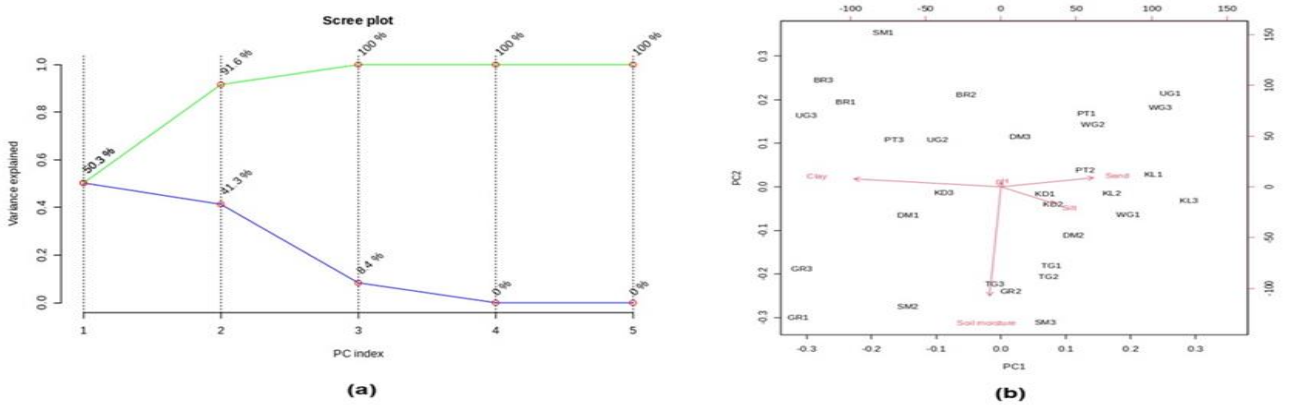

Figure 2: (a) Scree plot; (b) Bi-plot PCA Analysis of environmental factors in sample locations.

The soil in which *C. aeruginosa* grows has quite a large content of sand and silt, and it was found in 3 locations in Wonogiri, namely WG1, WG2, and WG3. Clay soil, a balanced mixture of sand, silt, and clay, has considerable potential for CO_2 absorption. Clay soils tend to foster an environment conducive to biological activity, facilitating the decomposition of organic matter, which contributes significantly to carbon storage. Clay soil, due to its fine texture and high mineral content, can store very large amounts of CO_2.[22] It indicates that clay particles have a large surface and can hold more organic materials. Meanwhile, the locations that contain silt and sand at the same ratio are found in 3 locations at Klaten, namely KL1, KL2, and KL3. As the topography of these locations is an area on the slopes of Mount Merapi, it has an impact on the spread of volcanic ash, causing the soil texture to be fine, silty, or sandy. Figure 3 shows variable importance projection (VIP) scores of PLS-DA of environmental variations in the location samples. The sand and clay make the greatest contribution to the location where CA-EOs grow, while other parameters such as pH, soil moisture, and silt did not contribute significantly.[23] From the VIP scores, the height of the growing location has a VIP>1 value which can be understood that height has the greatest contribution to the growing location. Figure 4 shows the scree plot and biplot PCA of yield and Micronutrient contents in soil. The sample location is connected to the yield and soil micronutrient variables, indicating that the sample location having high yield is KD1 and high organic carbon (OC) at the sample location is UG1. Yield and OC strongly correlate to PC 1 and PC2 in PCA. Figure 5 shows that yield and OC have VIP values > 1. Oldfield *et al*.[24] reported that there is a positive correlation between good agricultural land management and high OC content so the yield of EOs obtained is high.[22] The yield of EOs is influenced by soil type. Soil with a higher percentage of silt has a higher yield of EOs because silty soil has greater nutrients than sandy soil. The silty soil has a higher percentage of OC content, therefore, the production of EOs in a plant is greater. Aboukhalid *et al*.[10] reported that plants containing aromatic compounds have higher yields in calcareous soil than those on sandy soil. The yield of EOs from plants depends on various factors, such as soil texture, growing conditions, environment, and interactions with neighbouring plants.[25] These growth conditions are related to water availability, soil nutrients, sunlight, and environmental temperature.[26] Extreme and stressful ecological conditions, such as drought and excess mineral salts, affect the production of EOs.[27] Kome *et al*.[28] reported that soil pH is one of the main factors controlling soil fertility and plant nutrition because it affects the soil's ability to process the exact ratio of nutrients needed by a growing plant. Oil pH has an impact on a plant's ability to grow because it affects the availability of essential nutrients.[29-30] Plants mostly absorb the plant nutrients (N, P, and K) in which the soil with a lighter texture, such as sandy soil, tends to have lower nitrogen availability and cation exchange capacity compared to heavier soil, such as clay soil.[31] Plants absorb P element in the soil relatively less than the elements of N and K because P nutrient requirements of plants are generally less than those of elements N and K. The study of soil fertility status based on experimental results that the availability of phosphorus in the soil is very closely related to the acidity (pH) of the soil; in most soils, the maximum P availability is found in the pH range between 6.0-7.0, the availability of P will decrease if the soil pH is higher, lower than 6.0 or higher than 7.0.[27]

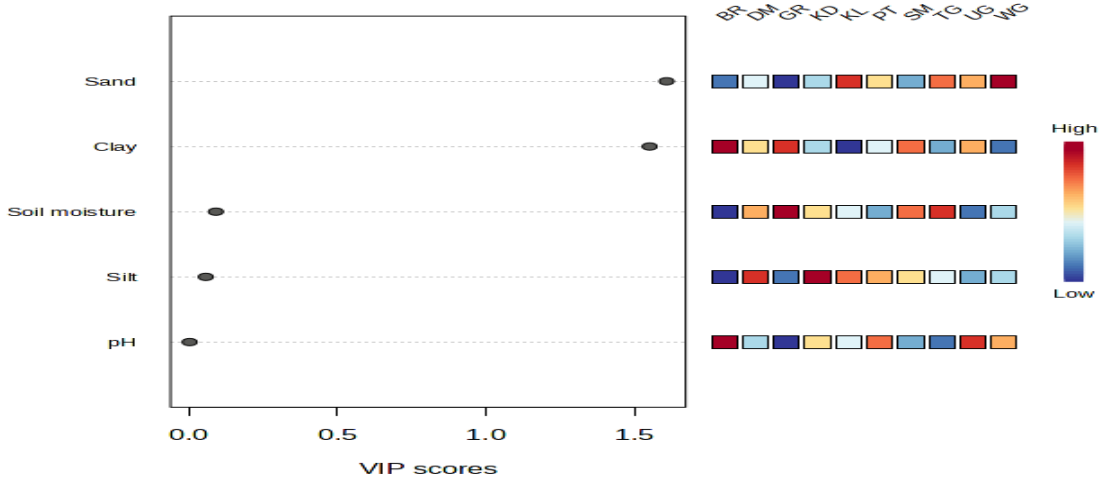

Figure 3: VIP scores of PLS-DA analysis of environmental factors in samples location

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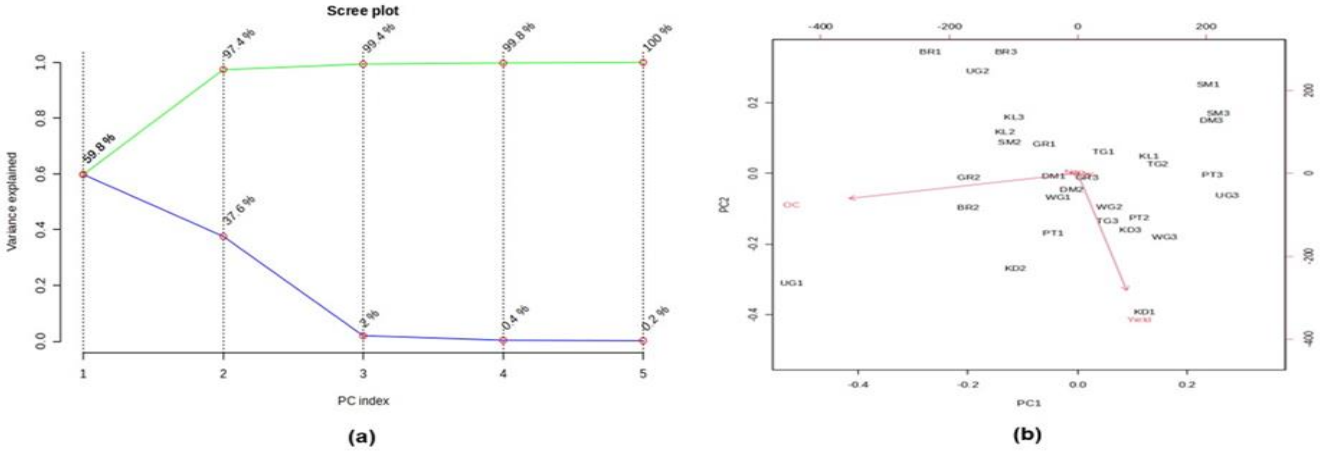

Figure 4: (a) Scree plot; (b) Biplot PCA Analysis of yield and Micronutrient content in soil

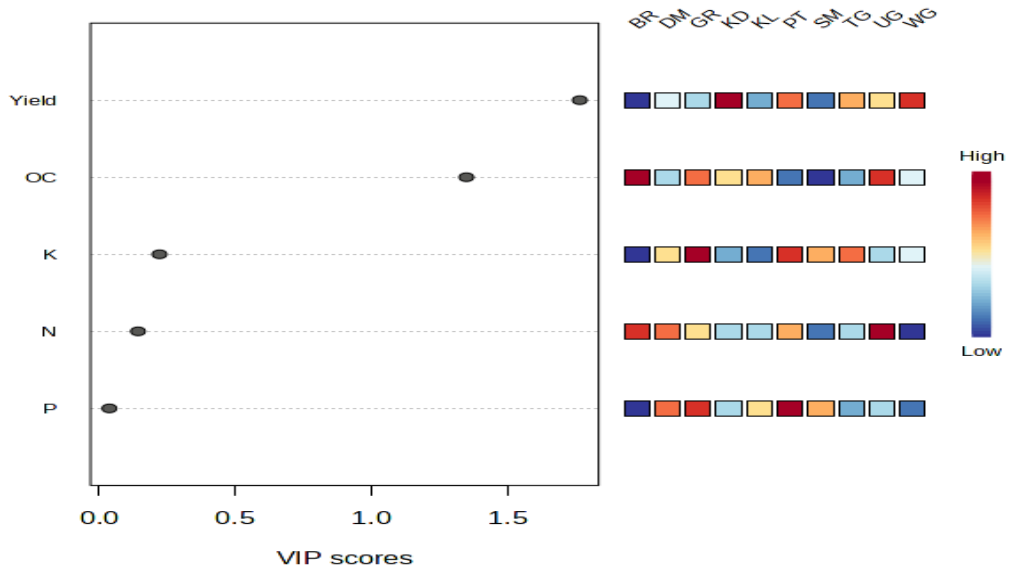

Figure 5: VIP scores of PLS-DA analysis of yield and Micronutrient contents in soil

The distillation process was carried out to obtain EOs contained in the rhizomes of *C. aeruginosa*. The steam-water distillation method was chosen because it can extract a lot of EOs. The steam-water distillation method uses a high-pressure steam system and raw materials that are not in direct contact with water. The steam flows through the container containing the raw materials. The steam coming out of the container is connected to a condenser so that the condensate liquid containing oil and water is separated according to the specific gravity of the oil. In this study, the yield of CA-EOs found in the UG2 location of the Semarang district was the highest (0.290% $^{v}/_{w}$). According to the Herbal Pharmacopoeia 2nd edition, the CA-EOs must contain not less than 1.89% $^{v}/_{w}$.[32] CA-EOs from 30 different growing locations have EO levels below 1.89% $^{v}/_{w}$. The different soil types and growing heights of *C. aeruginosa* plants can affect oil yield. GC-MS method was used to determine the oil contents of CA-EOs rhizomes. Each CA-EO content in the 30 location samples produces similar contents but with varying levels. Differences in the levels of EO contents of *C. aeruginosa* are influenced by the environmental conditions where *C. aeruginosa* plants grow, including weather, soil height, soil type, soil pH, temperature, and soil moisture, thus influencing the levels of plant metabolite content.[33]

Table 1 compiled the EOs contents of *C. aeruginosa* rhizomes in 30 locations samples in Central Java, mainly sesquiterpene compounds (curzerenone and germacrone) (Figure 6). Plants with the phytochemicals of curzerenone and germacrone were also found in *C. zedoria*,[34] *C. amanda*,[35] *C. aromatica*,[36,] and *Zingiber zerumbet*.[37] The identified compounds contained in the EOs of *C. zedoria* (Christm.) Roscoe using GC-MS are curzerenone and germacrone accounting for 22.30% and 9.0%, respectively.[38] *In vitro* study showed that curzerenone has strong antibacterial activity, especially for *Escherichia coli*, and strong antioxidant activity as assessed using beta carotene bleaching assay, reducing power, DPPH radical scavenging assay, and lipid peroxidation inhibition.[39] *In vitro*, study also indicated that curzerenone exhibited moderate to strong cytotoxic activity against Ca Ski and MRC-5.[40] Figure 7 shows the relationship between EOs, environmental factor variables, and soil micronutrients. Figure 7a illustrates the different images of the score plot, where the PCs selected by PLS-DA are orthogonal to those selected by PCA. The most important variable contributing to the PLS-DA model for the relationship between the sample locations with the chemical compounds of CA-EOs, and soil micronutrients was curzerenone (Figure 7b).

Table 1: The identity of eight essential oils from *C. aeruginosa* rhizomes at 30 locations as determined using GC-MS

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No	Samples Location	beta-Elemene	Iso-sericenine	Curzerenone	gamma elemene	Patchulane	Spathulenol	Germacrone	Calarene
1	TG 1	2.19	2.28	33.56	0.19	2.31	0.54	1.31	2.81
2	TG 2	1.68	1.57	21.96	0.13	1.20	0.20	0.94	2.00
3	TR 3	2.11	1.84	27.89	0.15	1.61	0.35	0.90	2.74
4	BR 1	1.58	3.10	41.22	0.22	0.13	1.12	5.67	1.60
5	BR 2	1.90	3.91	40.44	3.58	0.13	0.91	5.85	1.93
6	BR 3	1.78	3.45	43.73	0.24	0.15	0.93	4.89	1.72
7	WG 1	1.79	3.31	43.77	3.50	0.10	0.88	4.99	0.44
8	WG 2	1.39	3.08	37.89	0.27	0.15	1.20	6.55	1.34
9	WG 3	2.00	3.62	41.20	0.33	0.30	1.13	5.94	0.59
10	KD 1	1.34	3.13	41.71	4.23	0.29	1.27	6.12	0.39
11	KD 2	1.19	2.97	42.00	4.10	0.10	1.02	5.52	0.35
12	KD 3	1.67	3.38	40.70	4.19	0.31	1.12	6.10	0.49
13	UG 1	1.45	3.48	44.30	4.25	0.35	1.16	5.93	2.04
14	UG 2	1.65	3.69	51.92	3.51	0.38	1.12	3.65	1.75
15	UG 3	1.24	2.59	44.29	4.09	0.38	0.88	5.49	0.28
16	GR 1	1.67	2.14	39.73	0.18	2.91	0.65	1.57	3.41
17	GR 2	1.68	2.43	44.07	0.19	2.72	0.68	1.46	3.18
18	GR 3	1.58	1.98	31.37	0.38	2.48	0.62	1.36	2.93
19	KL 1	1.96	2.10	39.09	0.19	2.66	0.36	1.30	2.98
20	KL 2	1.83	2.42	42.75	0.19	3.08	0.70	1.53	2.79
21	KL 3	1.78	2.17	41.18	0.18	2.60	0.60	1.32	2.41
22	SM 1	2.35	2.55	42.36	0.20	2.86	0.62	1.60	3.97
23	SM 2	1.78	2.32	38.44	0.20	2.90	0.66	1.60	2.90
24	SM 3	1.55	1.97	35.77	0.16	2.31	0.55	1.31	2.54
25	DM 1	1.99	2.44	43.12	0.19	2.69	0.67	1.53	3.14
26	DM 2	1.83	2.18	29.34	0.18	2.51	0.60	1.34	2.91
27	DM 3	2.05	2.16	35.13	1.02	2.15	0.54	1.38	2.73
28	PT 1	1.40	3.42	42.22	4.03	0.35	1.21	6.50	0.38
29	PT 2	1.79	2.05	34.79	1.02	2.65	0.68	1.47	2.70
30	PT 3	0.80	2.76	49.22	3.26	0.31	1.00	5.24	2.27

The sample locations along with are TG1 = Clirit, Tegal; TG 2 = Kebonagung, Tegal ; TG 3 = Kalibakung tourism site, Tegal); BR 1 = Jatimulya, Brebes; BR 2 = Kubangjaya, Brebes; BR 3 = Kedung panjang, Brebes; WG 1 = Tanen, Wonogiri; WG 2 = Gedawung, Wonogiri; WG 3 = Gadungan, Wonogiri; KD 1 = Kedungsuren, Kendal; KD 2 = Darupono Field, Kendal; KD 3 = Darupono Forest, Kendal; UG 1 = BPTP-Gedang Anak, Ungaran; UG 2 = Sidomulyo, Ungaran; UG 3 = Bangka, Gedang Anak, Ungaran; GR 1 = Karangrayung, Grobogan; GR 2 = Penawangan, Grobogan; GR 3 = Godong, Grobogan; KL 1 = Bayat, Klaten; KL 2 = Karangpakel, Klaten; KL 3 = Trucuk, Klaten; SM 1 = Gunung Pati, Semarang, SM 2 = Blancir, Semarang; SM 3 = Pucung, Semarang; DM 1 = Mranggen, Demak; DM 2 = Pucang Gading, Demak; DM 3 = Jamus, Demak; PT 1 = Trangkil, Pati; PT 2 = Sukolilo, Pati; and PT 3 = Tlogowungu, Pati.

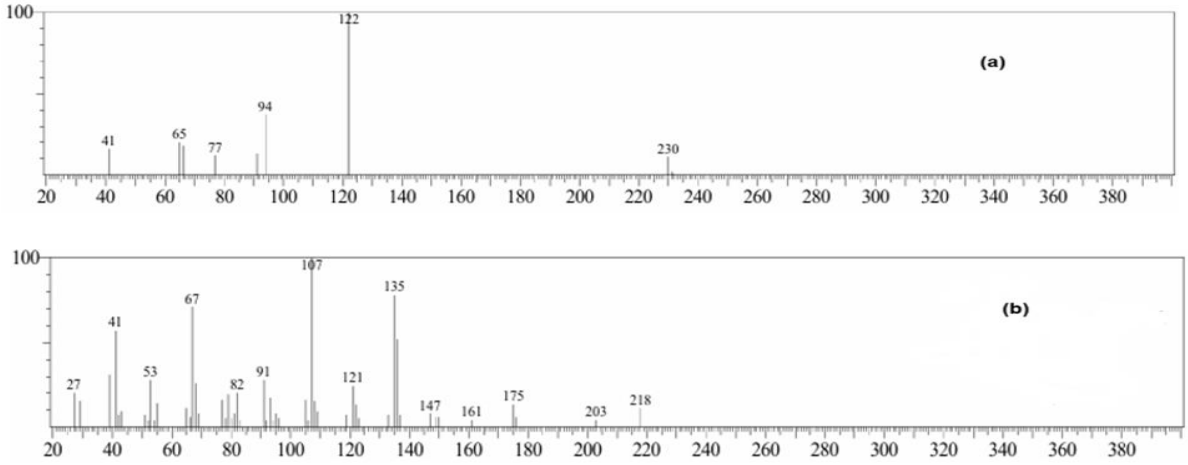

Figure 6: The identification Result of GC-MS in CA-EOs: (a) Curzerenone and (b) germacrone

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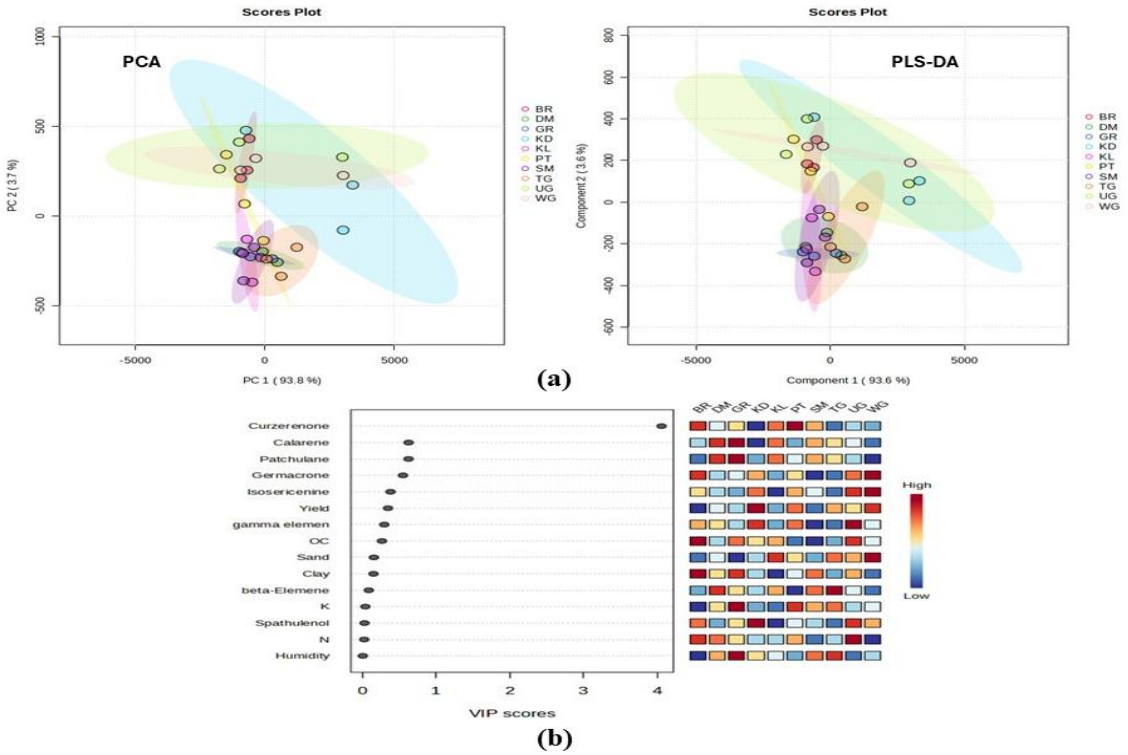

Figure 7: (a) PC in PCA and PLS-DA; (b) VIP scores of PLS-DA analysis of active component EOs *C. aeruginosa* with environmental factor and Micronutrient content soil

Conclusion

Various environmental and edaphic factors, as well as soil micronutrient content, influence the chemical content of CA-EOs. Sand, clay, organic carbon, and yield are variables that have an important contribution to the location of *C. aeruginosa* samples. Curzerenone shows high variability in the location of *C. aeruginosa* growth. The chemometric approaches are essential in understanding the complexity of the relationship between essential oils, environmental and edaphic factors, as well as soil micronutrient content. This chemometric approach plays an important role in the development of effective and sustainable essential oil production management strategies.

Conflict of Interest

The authors have declared no conflict of interest.

Authors' Declaration

The authors declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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