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Chemometrics-Assisted Fingerprinting Profiling of Extract Variation From Pagoda (*Clerodendrum Paniculatum* L.) Using Tlc-Densitometric Method

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Abstract

The application of chemometrics in the analyzing of chemical data from natural materials is essential in understanding complex chemical data. This study aims to apply the chemometric techniques of principal component analysis (PCA) and cluster observation (CA) for assisting the fingerprint profiling from 27 types of extracts from pagoda (*Clerodendrum paniculatum* L.) extraction using variations of solvent types (methanol, hexane, and ethanol), extraction techniques (maceration, Reflux, microwave-assisted extraction (MAE), and plant parts (flowers, leaves, and stems) which represent parts of plants. Each extract was subjected to thin-layer chromatography (TLC) using the mobile phase of hexane and ethyl acetate, and the measured Rf values of each spots obtained from densitometric evaluation at wavelength 254 nm was subjected to chemometrics analysis. The results exhibited that PCA showed variations in analyzed data describing more than 80% variances using thee principle components (PCs). Cluster analysis exhibited that all variables could be grouped into 10 clusters with the range of similarity indexes of 48.93%-99.78%. It can be concluded that chemometrics-assisted fingerprinting profile could differentiate the variations of Pagoda plant extracts according to solvent types, extraction techniques and parts of plants.

Keywords: Chemometrics, PCA, Cluster, Fingerprinting profiling, Pagoda, TLC-Densitometric.

1. Introduction

Chemometrics is a multidisciplinary study concerning with the application of mathematical and statistical methods for chemical data analysis. This technique requires the use of multivariate data analysis obtained from the measurement of chemical responses generated from sophisticated instruments including spectrophotometer and chromatograph [1,2]. With chemometrics, more information from chemical data can be retrieved, the measurement process can be improved and the useful information can be extracted from chemical and physical measurement [3-7]. The International Chemometrics Society (ICS) defined that chemometrics is the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods [8]. The concept of chemometrics can be used in experimental design, the relationship between independent and dependent variable using multivariate data, pattern recognition and multivariate calibration [9].

The application of chemometrics combined with some analytical methods including chromatographic and spectroscopic is common. This combination is successfully applied in multicomponent analysis using multivariate calibration [10,11] pattern recognitions either unsupervised such as principal

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component analysis (PCA) and supervised pattern recognition such as discriminant analysis. Thin layer chromatography-Densitometry is one of chromatographic techniques commonly used for separation of component in plant materials due to its implicitly and lower cost [12]. The compounds are measured based on spot/band image analysis obtained from TLC plates representing Rf values and the measured absorbance values, which can be extracted as a two-column matrix for chemometric analysis make this instrument most commonly used for current densitometric evaluation [12,13].

Pagoda (*Clerodendrum paniculatum* L.) is one of plant species among 560 others in genus Clerodendrum traditionally used in herbal medicine, especially in South Sulawesi, Indonesia. Empirically, Pagoda is used as antipyretic and anti-inflammatory [14]. The flower and leaf have been reported to treat hemorrhoids in northern Thailand [15]. The roots are also used as a traditional treatment of typhoid fever [16,17]. The roots and leaves are also used to treat rheumatism, antipyretics, anthelmintic, venereal diseases, and malaria, eye pain treatment, treatment of ailing, body aches, snake bites, and dizziness [18-22]. From literature review, there has been no research regarding the use of chemometric analysis on variables obtained from TLC-densitometric measurements of Pagoda. The objective of this study was to apply some chemometrics techniques namely principal component analysis (PCA) and cluster analysis of pagoda plant parts *Clerodendrum paniculatum* L. using some variables due to different solvents and extraction methods.

Experimental

Materials

The parts of Pagoda plants namely flowers, leaves, and stems are taken from Masamba City, North Luwu Regency, South Sulawesi Province, Indonesia. The plants were washed with running water, wet sorted, cut into small pieces, and then dried in a 50oC oven. The plant was dried and stored in containers using silica gel.

Preparation Extraction

The dried Pagoda plant samples in the form of flowers, leaves, and stems were accurately weighed of approximately 25 g and subjected to extraction. The extracting solvents used were methanol, ethanol 96%, and hexane with a ratio of 10:1 (w/v) between the sample and solvent. Three extraction methods were used, namely (1) maceration method for 24 hours with stirring 1-3 times, (2) microwave-assisted extraction (MAE) technique using power 30 watts for 19-minute, (3) reflux technique for 19 min. The extract solutions were then filtered using Whatman paper number 42 assisted by vacuum pump. Twenty-seven (27) samples were obtained from the combination of plant parts (flowers, leaves, and stems) and extraction methods (maceration, MAE, and reflux). The samples were (1) maceration hexane leaf, (2) Microwave hexane leaf, (3) Reflux hexane leaf, (4) Maceration hexane stem, (5) Microwave hexane stem, (6) Reflux hexane stem, (7) Maceration hexane flower, (8) Microwave hexane flower, (9) Reflux hexane flower, (10) Maceration methanol leaf, (11) Microwave methanol leaf, (12) Reflux methanol leaf, (13) Maceration methanol stem, (14) MAE methanol stem, (1% Reflux of methanol stem, (16) Maceration hexane flower, (17) MAE methanol flower, (18) Reflux methanol flower, (19) MAE ethanol leaf, (20) MAE ethanol leaf, (21) Reflux ethanol leaf, (22) Maceration ethanol stem, (23) MAE ethanol stem, (24) Reflux ethanol stem, (25) Maceration ethanol flower, (26) MAE ethanol flower and (27) Reflux ethanol flower.

TLC Densitometry Analysis

Twenty-seven extract solutions coming from the combination of plant part of pagoda plant, extraction techniques, and solvent types were spotted on TLC plate (silica gel 60 PF 254 size 20 cm x 20 cm) with lower limit of 1 cm and upper limit of 0.5 cm and distance of each extract 2 cm. The spots were eluted using three mobile phase systems consisting of hexane: ethyl acetate (5:1), hexane: ethyl acetate (2:1), and hexane: ethyl acetate (3:2). Furthermore, TLC plate was subjected into Densitometric measurement (CAMAG TLC Scanner) using software of 3 winCATS Planar chromatography analysis program, and the peaks from TLC chromatograms were subjected to chemometrics analysis.

Chemometrics analysis

The values of Rf (Retardation factor) and peak area of each peaks obtained from TLC-densitometry of 27 samples coming from the combination of plant

part and extraction techniques were analyzed Principal Component Analysis (PCA) and Cluster analysis (CA). The software Minitab® version 18 statistically using chemometric analysis using (Minitab Incorporation, USA) was used for performing chemometrics analysis.

Results and Discussion

Principal component analysis

Principal component analysis (PCA) is exploratory data analysis and is considered as one of unsupervised pattern recognition, typically used for classification of samples. In this study, the parts of dried Pagoda plant samples in the form of flowers, leaves, and stems extracted with three different techniques (maceration, microwave-assisted extraction and reflux), were subjected to PCA. There are twenty-seven extracts to be treated with chemometrics analysis (PCA and cluster analysis). All extracts were subjected to thin layer chromatography (TLC) systems and followed by densitometric analysis to get retardation factors (Rf) and peak area. Figure 1 revealed TLC-densitogram of Pagoda plants extracted with hexane, methanol and ethanol.

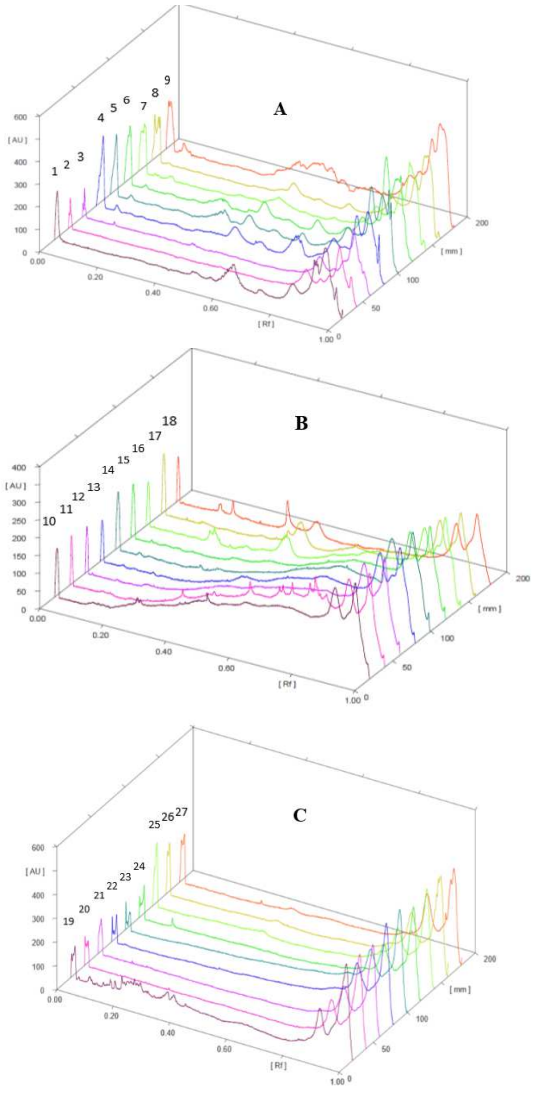

Figure 1: TLC-densitogram of Pagoda plant extracts, extracted by hexane [A], methanol [B] and ethanol [C]. For TLC-densitometry condition and identification of 1, 2, ... 27, *see Section of Experimental procedure*.

The Rf-values and peak area were used as variables during PCA and the score plot obtained from the correlation between the first principal component (PC1) and second principal component (PC2) was depicted in Figure 2. PC1 accounts for the most variation among the data, and PC2 accounts for next large variation. PC1 and PC2 accounts for 48.3% and 24.4%, respectively; therefore, two PCs contributed to extract 72.7% data variation. Figure 3 revealed the loading plot of PCA which described the contribution of each variable toward PC1 and PC2. If the data follows a normal distribution, and no outlier is present, then each variable will be randomly distributed around zero [23]. In loading plot, the further away from where the variable is placed, the higher the contribution of that variable to the PCA model [24].

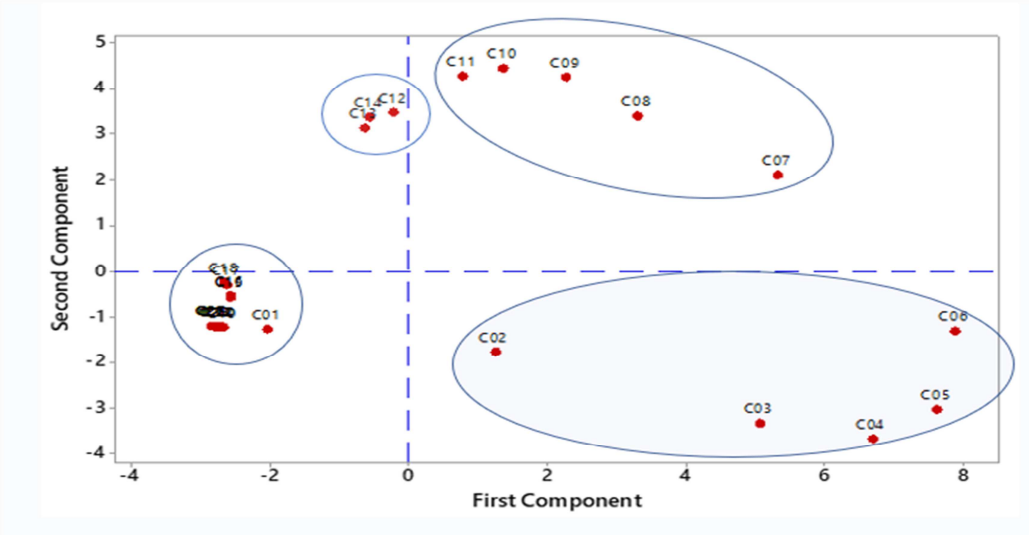

Figure 2: The sore plot for classification of samples using principal component analysis. For TLC-densitometry condition and identification of C1 (substance 1), C2 (substance 2), … C28 (substance 28).

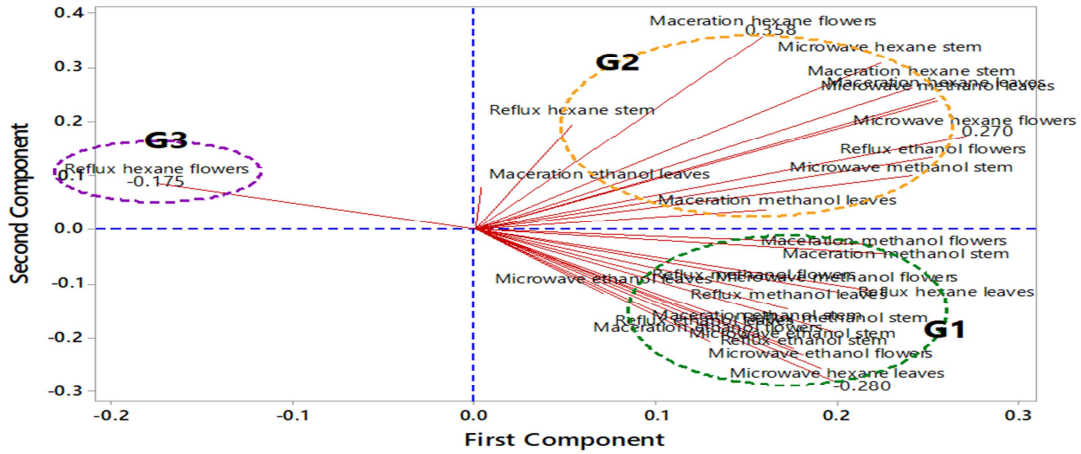

Figure 3: The loading plot describing the contribution of variables during principal component analysis of samples as classified as group 1 (G1) assigned with green line, group 2 (G2) assigned with orange line and group 3 (G3) assigned with purple line

The loading plot of PCA also describe the distribution of each variable in PCA results. If variables were normally distributed and no outliers were detected, each variable distributed randomly around zero and this also illustrated the effects of each variable toward the principle components and its correlation to each other. The score plot contributed to each variable used for the classification of samples. The further the score plot from origin point, the higher the contribution of variable to PCA model. From Figure 3, sample of Pagoda part extracted using microwave technique in group 1 (G1) revealed that the flower part of Pagoda extracted using hexane assisted with microwave affected positive value on principle component 1 (PC1) with score value of 0.270. The flower part extracted using hexane using Reflux affected negative value on PC1 with score value of -0.175 as in group 3 (G3). In addition, the flower part extracted using maceration affected positively in second principle component (PC2) with score value of 0.358 which correlated with other samples extracted by maceration revealed positive correlation with equation of y = 1.7447x + 0.0317; R^2=0.9099 as shown in Figure 4 .

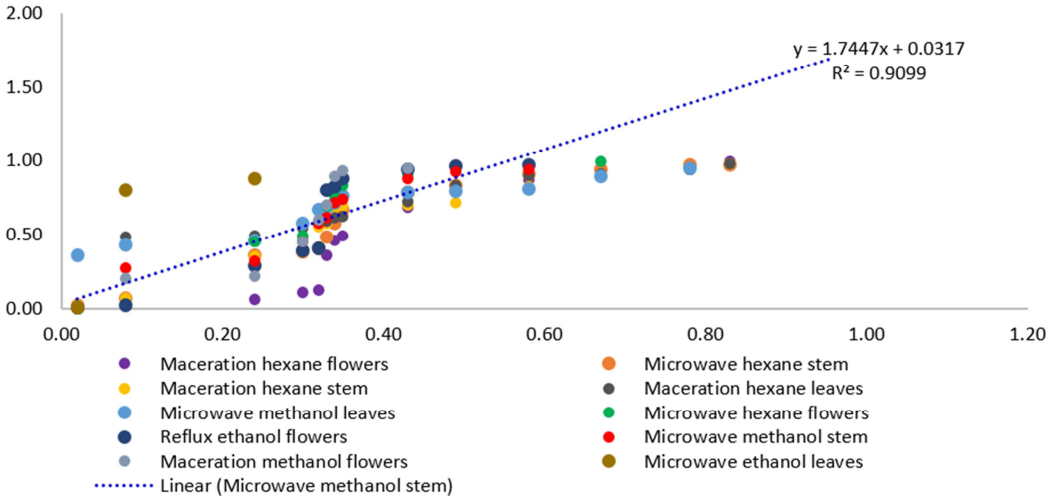

Figure 4: The second principal component (PC2) showing a positive correlation

Cluster analysis

Cluster analysis (CA) is one of unsupervised pattern recognition in which the variables and samples could be clustered based on Euclidean distance. **Figure 5** revealed the dendrogram obtained during clustering of variables namely Rf values of each spots present from TLC analysis of evaluated samples. From CA, the samples of dried Pagoda plant in the form of flowers, leaves, and stems extracted with three different techniques (maceration, microwave-assisted extraction and reflux) were classified and resulted 10 clusters with the similarity percentage of 48.93%-99.78% indicating that variables can be correlated with each other. Cluster 1 (C1) consisted of maceration hexane flower, maceration hexane stem, microwave hexane stem, maceration hexane leaf, and microwave methanol leaf, cluster 2 (C2) consisted of microwave hexane flower, microwave methanol stem, reflux ethanol flower, and maceration methanol leaf, cluster 3 (C3) consisted of reflux hexane flower, cluster 4 (C4) consisted of reflux hexane stem, and

maceration ethanol leaf, cluster 5 (C5) consisted of microwave hexane leaf, microwave ethanol flower, microwave ethanol stem, reflux ethanol stem, and maceration ethanol stem, cluster 6 (C6) consisted of reflux hexane leaf, microwave methanol flower, maceration methanol flower, and maceration methanol stem, cluster 7 (C7) consisted of reflux methanol flower, reflux methanol stem, and reflux methanol leaf, cluster 8 (C8) consisted of maceration methanol leaf, cluster 9 (C9) consisted of maceration ethanol flower and reflux ethanol leaf, cluster 10 (C10) consisted of microwave ethanol leaf.

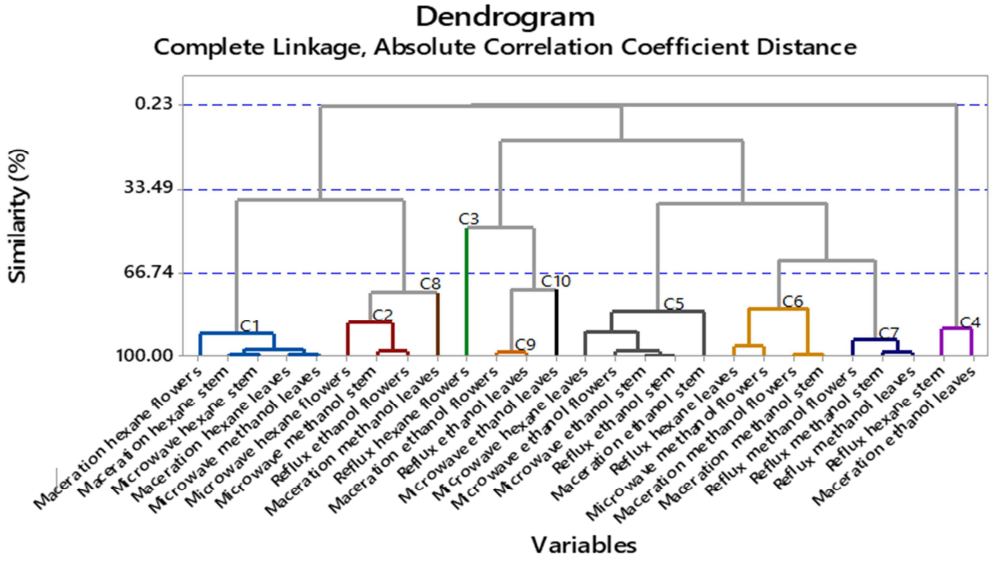

Figure 5: The dendrogram for classification of samples using cluster analysis. For members of each cluster C1, C2, …C10 assigned with the same colored line.

Conclusion

Principal Component Analysis (PCA) solvent variation and extraction method of pagoda plant extract in the form of flowers, stems, and leaves obtained 27 types of sections with eigenvalue scree plot value above 1 explaining 48.3% data, 0.67 explaining 72.7% of data, and 0.26 explaining 82.1% of data, Loading plot shows G1 group solvent hexane. Part interest microwave method shows a considerable positive influence on PC1 with a value of 0.270 and a mostly negative impact by solvent hexane, part reflux method with a value of -0.175 as the G3 group. In contrast, solvent hexane with the maceration method of the flower part has a large positive effect on PC2 with a value of 0.358, and solvent hexane, method microwave leaf part has an enormous negative impact on PC2 with a value of -0.280. The G1 and G2 groups showed a positive correlation with similarities between each variable. Cluster observation shows 10 clusters of variable

groups with %similarity ranging from 48.93% - 99.12%, and fingerprint shows there are 80 substance types detected at UV 254 nm with maximum absorption characteristics in the range of 200-700 nm with Rf values between 0.00-0.99, methanol solvent use saw 42 substance, ethanol solvent 37 substance, and hexane 57 substance of variation solvent and extraction method with different characteristics.

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Conflict of Interest

The authors have no conflict of interest.

Author Contributions

Budiman Yasir, Ayun Dwi Astuti, and Muhammad Raihan conducted the experiment, Subehan and Rosdiana Natzir conducted the calculations, Budiman Yasir, Gemini Alam, and Abdul Rohman wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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