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Determination of HPLC Chromatogram Profile of Katuk (*Breynia androgyna* (L.) Chakrab. & N.P.Balakr) Plants from Ristoja's Results using Chemometric Analysis

Penetapan Profil Kromatogram HPLC Tanaman Katuk (Breynia androgyna (L.) Chakrab. & N.P.Balakr) dari Hasil Ristoja Menggunakan Analisis Kemometrik

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Abstract

The katuk plant was known as *Sauropus androgynous* (L.) Merr, but the name is changed to *Breynia androgyna* (L.) Chakrab. & N.P. Balakr since the publication of Chakrab's & N.P. Balakr in the 2012 Journal of Plant Taxonomists. The content of chemical compounds and secondary metabolites of katuk leaf are strongly influenced by different habitat or location. Therefore, the Center for Research and Development of Medicinal Plants and Traditional Medicines, National Institute of Health Research and Development (NIHRD) has conducted Research on medicinal plants and herbs (RISTOJA) results on 58 samples of katuk plants originating from 7 provinces and used by 13 ethnic groups in Indonesia. This study aims to obtain plant quality based on the description of the High-Performance Liquid Chromatography (HPLC) chromatogram profile, in order to obtain the suitability of the efficacy of medicinal plants with their benefits for the community in an area. Test using HPLC with a gradient mobile phase, a mixture of acetonitrile and methanol for 60 minutes. The results of the chromatogram were analyzed chemometric by using Principal Component Analysis (PCA) data interpretation. PCA results showed that from HPLC chromatograms at 254 nm and 366 nm, each gave 3 different clusters, namely clusters A, B and C where each cluster has the same chromatogram profile of katuk plants. Cluster A which was identified at a wavelength of 254 nm was the most used cluster by 7 ethnic groups with a total of 28 katuk samples. Similarly, the chromatogram at a wavelength of 366 nm with the largest cluster is cluster A which is used by 11 ethnic groups with a total of 45 samples of katuk plants. where each cluster had the same chromatogram profile of katuk Plants.

Abstrak

Tanaman katuk dikenal sebagai Sauropus androgynous (L.) Merr, namun namanya diubah menjadi Breynia androgyna (L.) Chakrab. & N.P. Balakr sejak publikasi Chakrab's & N.P. Balakr di Jurnal Taksonomi Tumbuhan 2012. Kandungan senyawa kimia dan metabolit sekunder daun katuk sangat dipengaruhi oleh habitat atau lokasi tumbuh yang berbeda. Oleh karena itu, Pusat Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B_2P_2TOOT), Badan Penelitian dan Pengembangan Kesehatan (Balitbangkes) telah melakukan Riset Tanaman Obat dan Jamu (RISTOJA) untuk tanaman katuk sebanyak 58 sampel yang berasal dari 7 provinsi dan digunakan oleh 13 etnis di Indonesia. Penelitian ini bertujuan untuk memperoleh mutu tanaman berdasarkan gambaran profil kromatogram High Performance Liquid Chromatography (HPLC), guna mendapatkan kesesuaian khasiat tanaman obat dengan kemanfaatannya untuk masyarakat di suatu daerah. Pengujiaan menggunakan HPLC dengan fase gerak gradien, campuran asetonitril dan metanol selama 60 menit. Hasil kromatogram HPLC dianalisis secara kemometrik dengan menggunakan interpretasi data Principal Component Analysis (PCA). Hasil PCA menunjukkan bahwa dari kromatogram HPLC pada 254 nm dan 366 nm, masing-masing memberikan 3 kluster yang berbeda yaitu kluster A, B dan C dimana tiap kluster mempunyai profil kromatogram tanaman katuk yang sama. Kluster A yang diidentifikasi pada panjang gelombang 254 nm merupakan kluster terbanyak digunakan oleh 7 etnis dengan jumlah 28 sampel katuk. Demikian pula kromatogram pada panjang gelombang 366 nm dengan kluster terbanyak adalah kluster A yang digunakan oleh 11 etnis dengan jumlah 45 sampel tanaman katuk.

INTRODUCTION

Utilization of katuk leaves in Indonesia is widely consumed by mothers who have given birth to increase milk production, because of the high nutritional content of katuk leaves.[1,2,3,4] Based on the results of molecular studies by T. Chakrabarty and N.P. Balakrishnan in 2012 published in the Journal Plant Taxon 2012 (December) stated that the name *Sauropus androgynous* became *Breynia androgyna* (L.) Chakrab. & N.P. Balakr.[5] However, until now there are still many researchers who do not know about the name change, it is proven that there are still many publications that use *Sauropus androgynous* for the katuk plant.[2,6,7] The part of the katuk plant that is commonly used is the leaf. Chemical compounds contained in katuk leaves can vary depending on the type of extraction, harvest time, place of growth and climate (weather), beside that the drying process will affect the levels and composition of compounds contained in the plant.[8] Several drying methods on herbs are carried out to obtain optimal content, and the water content is reduced so that it can be stored for a long time. Drying is done, among others, using the sun, oven or freeze-drying and aerated.

In the cinnamomum plant which was extracted with methanol and water, it was found that the sun-dried samples had degraded total phenolics and flavonoids compared to fresh samples.[8] Research using air drying compared to using oven drying gave higher total phenolic content, antioxidant activity and flavonoids than using oven drying.[9]

Katuk leaves have been widely studied for their chemical content, ethanol extract of katuk leaves contains alkaloids, flavonoids, terpenoids, tannins, glycosides.[10] Alkaloid and sterol compounds are thought to have effectiveness on breast milk production.[11] One of the effects of the content of secondary metabolites is the place of growth. This is one of the objectives of Research on Medicinal Plants and Herbs (Ristoja) conducting research on medicinal plants in several provinces in Indonesia. One of the plants that Ristoja sampled was the katuk plant. Ristoja's research samples came from various regions in Indonesia. The distribution of the katuk plant from Ristoja comes from 13 ethnic groups in the territory of Indonesia, which come from 7 provinces including West Kalimantan, Maluku, NTB, NTT, Papua, West Papua, Central Sulawesi.

To determine Chromatogram profile of Katuk leaves from various regions in Indonesia, the chemical content of katuk leaves was measured using the High Performance Liquid Chromatography (HPLC) method. Measurement conditions using a PDA detector, column C18 column 5.0 um x 4.6 x150 mm, flow rate 1.0 ml/min to obtain intensity data for each test sample and analyzed using the chemometric method. The data obtained are grouped based on the tendency of the similarity of chemical compounds, then from the results obtained it will be possible to know the chemical profile of katuk leaves from Ristoja's results which are spread in several regions in Indonesia.

Chemometric analysis is a statistical and mathematical science that is used to process chemical data. While Principal Componenet Analysis (PCA) is used for grouping data, looking at the quality diversity of different geographical conditions

Therefore, it is necessary to determine the profile of the Katuk plant as a result of Ristoja's research from 7 provinces consisting of 13 ethnicities.As Nuryani's research conducted control of the quality of orthosiphon leaves on the diversity of 3 regions that have different geographies, using PCA and Partial Least Squares Discriminant Analysis (PLSDA) chemometric analysis.[12]

METHOD

Material and Equipment

The material to be tested is katuk leaf (*Breynia androgyna* (L.) Chakrab. & N.P. Balakr) obtained from RISTOJA in 7 provinces (13 ethnicities) of Indonesia (NTT, NTB, West Kalimantan, Central Sulawesi, Maluku, West Papua, and Papua). The solvents used were methanol HPLC grade (Merck, Germany) distilled water (IKA), HPLC grade acetonitrile (Merck, Germany), andrographolide standard (Sigma-Aldrich)

Equipment. One set of HPLC (Waters 2695, detector PDA, column C18 5,0 um x 4,6x150 mm (Waters) analytical balance (Ohauus), oven (Memmert)

Preparation sample

The katuk plant to be tested is first determined in the laboratory of Medicinal Plant and Tradisional Medicine, Tawangmangu, Central Java.

Processing

Sample preparation

Katuk leaves (*Breynia androgyna* (L.) Chakrab. & N.P. Balakr) were separated from the twigs and cleaned of impurities, washed and dried in an oven at 50^{o} C for a day and night. The sample was made in powder form and then put 150 mg into a closed tube measuring 1.5 ml. and loaded into the Brospec Mini Beadbeater-16. The tool runs for 30 seconds 1-2 times, each time the tool is run for 30 seconds with a pause of 60 seconds until the test sample becomes a fine powder. The bead (gotri) is removed then the sample is stored and tightly closed.

Preparation of test solution

The test sample that has been in the form of a fine powder is weighed as much as 100 mg and put into a 25 ml capped bottle, then 10 ml of methanol is added, then close tightly and let stand overnight at room temperature protected from sunlight. Then the sample was filtered with a 0.45µm syringe filter and the filtrate was put into a closed microtube. Store at room temperature protected from light and HPLC examination was carried out before 24 hours.

Optimization and performance testing

Before starting HPLC measurements, it is necessary to optimize the gradient system, flow rate, injection volume and required elution time. Performance tests were carried out to ensure measurements with HPLC were running well. Performance tests were carried out using the Andrographolid standard. Andrographolid is not a compound present in the katuk plant, but the function of using the standard in this HPLC performance test is to ensure that the HPLC tool used is running well, with constant peaks produced at standard repetitions in every 10 test samples.

Measurement of test solution

Before measuring the test solution, we must measure the blank using methanol. The base line of the blank solution chromatogram must contain a signal <3x noise, where a blank measurement is performed every 10 injections of the test solution. This was done to ensure that the base line was maintained and standard Andrographolid injection was performed after every 10 injections of the test solution. Before the end of the test, it is necessary to flush with 100% distilled water for 15 minutes and 100% methanol/acetonitrile for 15 minutes.

Chemometric data analysis

Chromatogram data obtained from HPLC measurements are most often used to identify chemical compounds of many plants combined with chemometrics using Uncramble 9.7 Software and continued with *Principal Component Analysis* (PCA) analysis. PCA analysis is a method that is often used to process multivariate data with unknown samples, or a multiple variable analysis method that aims to simplify the observed variables by

shrinking (reducing) their dimensions. Chromatograms of katuk plants belonging to the same ethnic group were put together. If any chromatogram is very different from the other chromatograms, then the different chromatograms are excluded. Chromatogram mean yield for each ethnicity was analyzed by PCA. Next, grouping based on the tendency of PC1, PC2 and PC3 according to the obtained chromatogram uniformity.

RESULT AND DISSCUSION

Total samples are 58 samples from Ristoja's 2017, katuk leaf test were collected from 7 provinces in Indonesia. The local name of this katuk plant varies depending on the origin ethnicity where the sample comes from. Local names, origins and ethnicities where the katuk plant originates are presented in Table 1. In general, each ethnic group provides a test sample of 5 katuk plants, but there are also only 3, due to the limitations of the katuk plant in that ethnic group.

Optimization and performance testing

Optimization of the mobile phase using the standard Andrographolid to obtain optimum HPLC conditions was carried out starting at a wavelength of 200 nm to 500 nm. Measurements were carried out for 60 minutes using a PDA detector. Optimum injection volume was obtained at 10 ul, with a gradient mobile phase using a combination of methanol and acetonitrile solvents, injection volume 10.0 L, with a flow rate of 1.0 mL/minute, Optimization of the mobile phase at HPLC is presented in Table 2.

Table 1. Sample origin, ethnicity, local name and number of samples

No	Province	Etnicity	Local name	Number of samples
1.	NTT	Danggo	Kambesi	5
2.	NTB	Bajawa	Katu	5
3.	Kalimantan Barat	Mali	Cangok Manis	5
4.	Kalimantan Barat	Ngabang	Cangok Manis	5
5.	Kalimantan Barat	Sambas	Cangok Manis	5
6.	Sulawesi Tengah	Patinio	Cangok Manis	5
7.	Sulawesi Tengah	Rongkong	Katu'	5
8.	Maluku	Asilulu	Katok	3
9.	Maluku	Danar	Katuk	4
10.	Maluku	Fordata	Katok	5
11.	Papua Barat	Inawatan	Katuk	5
12.	Papua	Denta	Katuk	3
13.	Papua	Komoro	Katuk	3

Table 2. Standard andrograpolid optimization and performance test using HPLC

No.	Time (second)	Acetonitril (ml)	Metanol (ml)
1	0	50	50
2	1	50	50
3	30	50	50
4	40	20	80
5	60	20	80

Performance tests using Andragrapolid external standards were carried out on every 10 test samples to ensure that the measurements were still running well while still producing the same Andrographolid standard chromatograms. The unavailability of a standard which is the active substance of this katuk plant, so the Andrographolid standard is used to ensure that the test continues properly, seen from the stability of the resulting peak and the resulting peak is not tailing.[13,14] The HPLC chromatogram of the Andrographolid performance test results can be seen in Figure 1.

Measurement of test solution

Prior to the measurement, the eluent optimization must be carried out first to obtain the eluent composition and the time required for HPLC measurements. Optimization results were obtained using a combination of distilled water and methanol eluent with the following variations (Table 3.). The composition of the mobile phase obtained was then used to measure the katuk leaf test solution, which amounted to 58 pieces at a wavelength of 254 nm and 366 nm. An example of the chromatogram results of one of the test solutions on HPLC measurements at 254 nm and 366 nm can be seen in Figures 2 and 3. Based on the results of the HPLC chromatogram of katuk leaves, it was seen that measurements at a wavelength of 254 nm gave relatively fewer peaks compared to measurements at a wavelength of 366 nm. This shows, measurements at 366 nm more chemical compounds were detected at that wavelength than at 254 nm.

Table 3. Results of optimization of katuk samples measurements based on mobile phase gradient

No	Time (second)	Aquades (ml)	Metanol (ml)
1.	0	80	20
2.	2	70	30
3.	10	50	50
4.	23	30	70
5.	40	0	100
6.	45	80	20
7.	60	80	20

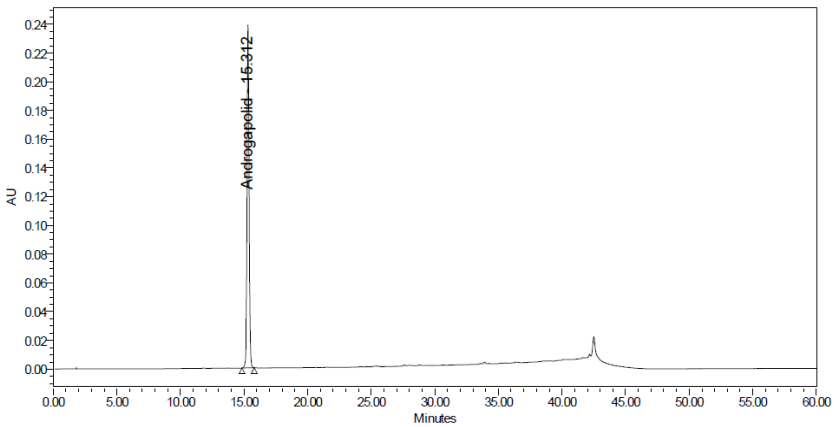

Figure 1. HPLC chromatogram on andrographolide performance test

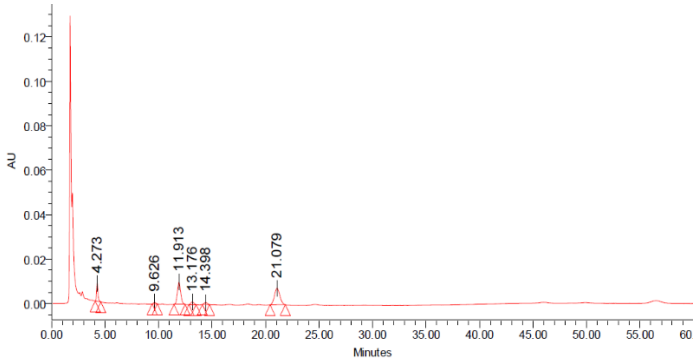

Figure 2. HPLC chromatogram katuk samples at a wavelength of 254 nm

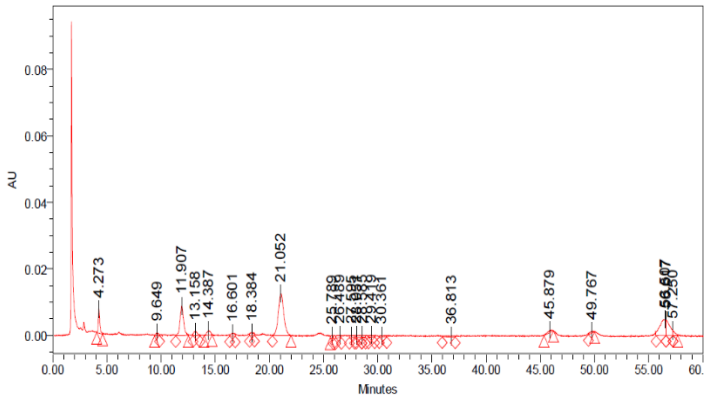

Figure 3. HPLC Chromatogram katuk samples at a wavelength of 366 nm

Principal Component Analysis yield of katuk plants at 254 nm. The intensity data on the HPLC chromatograms were analyzed chemometrically using Unscrambler software. To interpret the HPLC data, chemometric analysis was performed using PCA. The results of the HPLC intensity of each sample at 254 nm were converted into excel format and then continued with PCA analysis to obtain groupings / clusters, where objects that have similarities are in the same cluster, and those that do not have any resemblance at all will be in the same cluster. outside the cluster (outlier), where the samples in each cluster have the same chromatogram profile The PCA results from HPLC measurements at 254 nm were divided into 3 clusters (showing 3 chromatogram profile) (Figure 4), where the samples in each cluster were assumed to have the same smiliarity of chromatogram. Smiliarity of chromatogram is assumed to have the same quality. The PCA results at 254 nm illustrate that Ristoja's katuk plants have 3 chromatogram profile of katuk plants. Many chromatogram assessments or classifications have been carried out between plants with different geographical origins in the application of chemometric applications in plant quality control.

The identification of the chromatogram at a wavelength of 254 nm and the PCA results showed that cluster A was the ethnic group that used the most katuk samples. There are 5 ethnic groups, namely Donggo (NTT), Mali (West Kalimantan), Patinjo (Central Sulawesi), Rongkong (Central Sulawesi), Fordan (Maluku) each using 5 katuk samples for treatment, in addition there are 2 ethnicities namely Asilu (Maluku) and Inawatan (Papua barat) below 5 samples. Meanwhile, in Cluster B, there are 3 ethnic groups Bajawa (NTB), Ngabang (West Kalimantan) and Sambas (West

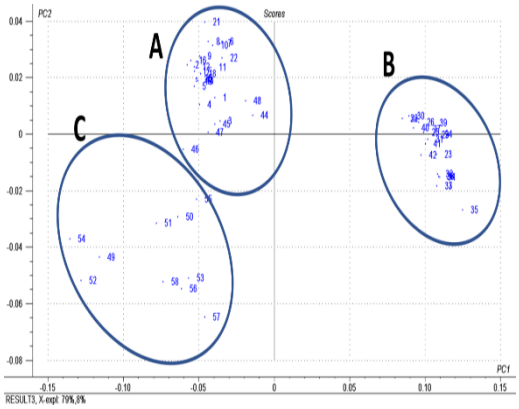

Figure 4. Clustering of katuk samples resulting from HPLC measurements at 254 nm

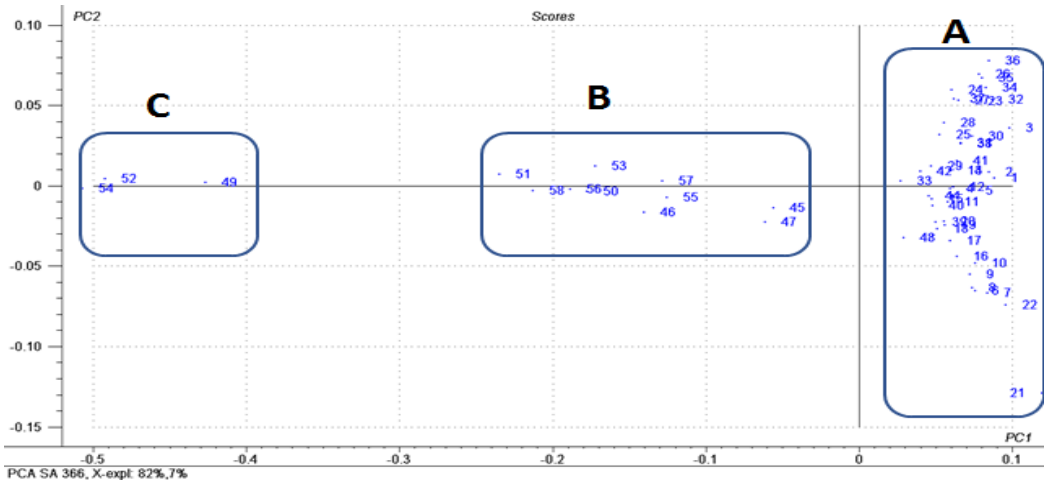

Figure 5. Clustering of katuk samples resulting from HPLC measurements at a wavelength of 366 nm

Kalimantan) which use 5 katuk samples each. For Cluster C, the katuk samples are used by the Innawatan (Pabar), Denta and Komro (Papua) ethnic groups. all three under 4 samples (Table 4).

The PCA results of katuk plants from Ristoja from various locations in 7 provinces at a measurement of 254 nm have 3 different chromatogram profile depicted in clusters A, B and C in Table 4. Elda researchers said that the differences in growing locations followed environmental differences can affect the quality of tobacco.[15] It is also seen in the research here that the influence of the location where the test sample grows can provide different qualities due to different environmental conditions. PCA results from HPLC chromatograms measured at a wavelength of 366 nm resulted in 3 clusters. Cluster A is the largest cluster with ethnic groups using katuk samples, which are 11 ethnicities. There are 7 ethnic groups (Donggo (NTT), Mali (West Kalimantan), Patinjo and Rangkong (Suteng), Asilu (Maluku) and Bajawa (NTB), each using 5 katuk samples. For Cluster B, the most ethnic groups who use katuk are 4 There are less than 5 ethnic groups using katuk, namely Fordata (Maluku), Innawatan, Dental, and Komro, all three are from Papua province, while in Cluster C there are only 2 ethnicities, namely Innawatan (Papua Barat) and Denta (Papua) with 2 and 1 katuk samples respectively (Table 4).

PCA analysis at 366 nm measurements gave 3 clusters (Table 4), each cluster has its own chemical content, where the chemical composition of each medicinal plant can be analyzed to identify medicinal plants to ensure the authenticity of the plant to prevent.[15,16] At this wavelength of 366 nm, the katuk plant produced by Ristoja has 3 qualities where the katuk plant of the same species may have different qualities and efficacy, this is in accordance with growing conditions such as soil, cultivation and climate based on differences in geographical origin.[17,18]

These results indicate that measurements at a wavelength of 254nm and 366 nm produce 3 clusters each, with the most being Cluster A, 7 ethnicities were found using 28 samples (254 nm), while cluster A, identified at a wavelength of 366 nm, was found in 11 ethnicities. using 45 samples of Katuk. Cluster C is the cluster that uses the fewest ethnicities and the number of samples, each with 3 ethnicities with a number of samples below 4 samples (254 nm) and 2 ethnicities with a number of samples below 2 (366 nm).

All PCA results from katuk plants at wavelengths of 254 and 366 nm were all included in each cluster, there were no outliers, as was Ristoja's results for ciplukan plants.[19]

Table 4. Grouping the results of PCA analysis based on HPLC chromatogram on the use of 58 katuk samples by 13 ethnicities

Cluster A		Cluster B		Cluster C	
	Number of samples		Number of Samples		Number of Samples
Wavelength of 254 nm					
Donggo (NTT)	5 sample	Bajawa (NTB)	5 sample	Innawatan (Pabar)	4 sample
Mali (Kalbar)	5 sample	Ngabang (Kalbar)	5 sample	Denta (Papua)	3 sample
Patinjo (Sulteng)	5 sample	Sambas (Kalbar)	5 sample	Komro (Papua)	3 sample
Rongkong (Sulteng)	5 sample	Asilu (Maluku)	1 sample		
Asilu (Maluku)	2 sample	Danar (Maluku)	4 sample		
Fordan (Maluku)	5 sample				
Innawatan (Pabar)	1 sampel				
Wavelength of 366 nm					
Donggo (NTT)	5 sample	Fordata (Maluku)	3 sample	Innawatan (Pabar)	2 sample
Mali (Kalbar)	5 sample	Innawatan (Papua)	2 sample	Denta (Papua)	1 sample
Patinjo (Sulteng)	5 sample	Dental (Papua)	2 sample		
Rangkong (Sulteng)	5 sample	Komro (Papua)	3 sample		
Asilu (Maluku)	3 sample				
Bajawa (NTB)	5 sample				
Ngabang (NTB)	5 sample				
Sambas (Kalbar)	5 sample				
Danar (Maluku)	4 sample				
Fordatal (Maluku)	2 sample				
Innawatan (Pabar)	1 sample				

CONCLUTION

Chemometric analysis with PCA can be used to ensure plant quality based on HPLC chromatogram data, so as to ensure efficacy in accordance with the use of plants for treatment to the community. Identification of the chromatogram at a wavelength of 234 nm resulted in 3 clusters, namely Cluster A, B, and C. Cluster A was the most used cluster by 7 ethnic groups with a total of 28 Katuk samples. Similarly, the chromatogram at a wavelength of 366 nm obtained 3 clusters, with the largest cluster being Cluster A which was used by 11 ethnic groups with 45 samples of Katuk plants.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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