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Authentication of *Drimys piperita* Hook f. Tree Bark Infusion from the Adulteration of *Cinnamomum burmannii* Nees Ex Bl. using the Combination of UV Spectroscopy and Chemometrics Techniques

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ABSTRACT: *This study aims to develop a method to authenticate akway bark (Drimys piperita Hook f.) infusion using UV spectroscopy and chemometrics techniques. The background initiative of this study is the consideration of the high economic value of akway bark which may potentially lead to the adulteration of the akway raw materials for traditional medicine. The sample used in this study was akway bark obtained from Manokwari, Papua, Indonesia. Samples of akway bark, cinnamon, and a mixture of both were prepared in the form of powder and then infused. The infusion was examined using UV spectroscopy to obtain the absorption value of each wavelength. Chemometrics techniques including principal component analysis (PCA) and multivariate calibration using principal component regression (PCR) and partial least squares (PLS) regression were carried out during the study. Additionally, computational discrimination using sparse partial least squares discriminant analysis (sPLS-DA) was performed afterwards. A total of 36 distinctive wavelengths were obtained. The absorption values were then used to form a PCA model. The best multivariate calibration model was derived from PCR data processing on the original spectra for both akway and cinnamon bark infusion samples. The AUC-ROC values obtained from the application of the sPLS-DA technique for each sample were 1.000, 0.956, and 0.633 for akway bark, cinnamon, and the mixture of both, respectively. Authentication of akway bark infusion has been successfully conducted on the presence of cinnamon as the adulterant.*

Keywords: *akway; authentication; chemometrics; Papua*

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1. Introduction

Akway (*Drimys piperita* Hook.f) is a plant endemic to Papua, Indonesia, which is a woody, aromatic-leaved plant classified into the Winteraceae family as shown in Figure 1 [1]. Akway grows naturally in the mountainous areas of West Papua specifically in Anggi district, Manokwari [2]. It is a wild plant used by the local community as a plant for traditional medicine. The locals use it as aphrodisiacs, tonics, antimalarials, and antioxidants [3–6]. They often use akway by scraping the bark, making it into powder, and infusing it with hot water. They drink the infusion or bite it during long journeys to increase endurance and stamina [7].

The high demand of akway and its limited availability in nature could potentially lead to adulteration [8]. Customers purchases akway in the market should be careful when buying the bark as it is organoleptically similar to cinnamon (*Cinnamomum burmannii* Nees Ex Bl.), where the colour and spicy taste of akway bark are identical to cinnamon [9].

Therefore, it is crucial to authenticate akway bark infusion. The authentication procedure was conducted using the UV spectroscopy method [10]. In this study, chemometrics techniques were mainly used to process the data due to its ability to combine statistics and mathematics in processing chemical data [11]. The chemometrics applied in this study included the exploratory data analysis, multivariate calibrations, and discrimination techniques. The exploratory data analysis technique used in this study was the principal component analysis (PCA). The multivariate calibration techniques of principal component regression (PCR) and partial least squares (PLS) regression were applied to different spectral types in order to achieve the best predictive model for quantifying the adulterant content. The discrimination technique of sparse partial least squares discriminant analysis (sPLS-DA) along with parameter evaluation was conducted to perform authentication analysis of *Drimys piperita* Hook.f [12].

The objective of this study is to develop a method to authenticate the infusion of akway bark (*Drimys piperita* Hook f.*)* on cinnamon as the adulterant using UV spectroscopy and chemometrics techniques. Further, this study expects to contribute to the effort on detecting the quality of Indonesia native raw materials, especially from Papua, which has not been widely explored.

2. Materials and methods

2.1. Materials

The materials used in this study were akway bark obtained from Arfak Mountains, Manok-



Figure 1. Documentation of the *Drimys piperita* Hook f. tree bark

wari, Papua, Indonesia, cinnamon obtained from a local market in Jayapura, Papua, Indonesia, and distilled water obtained from the Laboratory of the Faculty of Pharmacy at Sanata Dharma University, Indonesia.

2.2. Tools

Tools used in the study were UV-Vis spectroscopy of Shimadzu® UV-800 brand, R software (version 4.3.3) and RStudio (version 2024.09.01 Build 394), cuvette (Hellma Analytics®), semi-micro analytical balance Ohaus® (PAJ1003 series max. 120g; min. 0.001g), Buchner erlenmeyer, Buchner funnel, vacuum, 100-1000 µL micropipette (Acura® 825), blue tip, volume pipette (Pyrex®), alcohol thermometer, hot plate, volumetric flask (Pyrex®), dropper pipette, beaker glass (Pyrex®), stirring rod, and oven.

2.3. Methods

The method used in this study refers to the previous research conducted by Riswanto et al with some modifications [10]. The chemometrics techniques were implemented during the study to identify akway bark infusion against cinnamon as the adulterant.

2.3.1. Preparation of infusion samples of akway bark, cinnamon bark, and their mixture

Akway bark and cinnamon bark were dried and then processed into a powder. The akway powder was weighed 5.0 mg and prepared in five replications. The cinnamon powder was weighed 5.0 mg and prepared in five replications. Calibration and validation test samples containing akway bark powder and cinnamon powder were prepared from a mixture of these ingredients with a concentration of 10-90% (w/w) in 10% intervals thus obtaining nine variations of concentration of solutions. Three replications were made where the first and second replications were used as calibration solutions and the third was used as a validation solution. The details of the sample preparation were presented in Table 1. Each replication was infused with hot water at 90°C up to a volume of 200 mL. The duration of the infusion process was 5 minutes.

2.3.2. Sample preparation for discrimination

Solutions for sPLS-DA analysis were prepared according to Table 1. All the solutions were subsequently labelled to create a discrimination analysis model.

Table 1. Sample preparation of akway powder, cinnamon powder, and binary mixture containing akway and cinnamon powder

No	Labels	Weight of each component (g)		Number of replications
		Akway	Cinnamon	
1	A:C (100:0)	5.0	0.0	5
2	A:C (0:100)	0.0	5.0	5
3	A:C (10:90)	0.5	4.5	3
4	A:C (20:80)	1.0	4.0	3
5	A:C (30:70)	1.5	3.5	3
6	A:C (40:60)	2.0	3.0	3
7	A:C (50:50)	2.5	2.5	3
8	A: C (60:40)	3.0	2.0	3
9	A:C (70:30)	3.5	1.5	3
10	A:C (80:20)	4.0	1.0	3
11	A:C (90:10)	4.5	0.5	3

2.3.3. Data acquisition using UV Spectroscopy

UV spectra scanning was carried out on the infusions of akway bark, cinnamon, and a mixture of both. The distilled filtrate was then poured into a cuvette and scanned at a wavelength of 210-400 nm.

2.3.4. Exploratory data analysis using PCA

The UV spectra obtained from the spectra scanning stage were then evaluated. The dominant peaks from the analysis, namely akway bark, cinnamon, and samples of mixture containing both ingredients were utilised to build a PCA model. Scree plot, variable plot, and individual plot were displayed to visualize the model [13].

2.3.5. Multivariate calibration analysis

PCR and PLS multivariate calibration models were established to produce predictive models of the content of akway bark and cinnamon bark infusions. The performance of the multivariate calibration models was examined by evaluating statistical parameters including the coefficient of determination for calibration (R_{cal}^2), cross-validation (R_{cv}^2), validation (R_{val}^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP). The cross-validation process, as an internal validation, used the leave one-out technique. The multivariate calibration models selected for each filtrate were determined by evaluating R_{cal}^2, R_{cv}^2, R_{val}^2, RMSEC, and RMSEP [14].

2.3.6. sPLS-DA model development

The sPLS-DA model was produced using the UV spectra of akway bark, cinnamon, and samples of mixture containing both ingredients. Background prediction and 3D individual plot visualised the discrimination model. The model performance evaluation indicated the operational characteristic under curve-receiver (AUC-ROC) [15]. Further optimization of the model was carried out by selecting output variables until a final result that considered the error rate in classification and feature selection was obtained.

3. Results and discussion

This study developed a rapid detection technique for the adulteration of akway bark infusion using UV spectroscopy method combined with chemometrics techniques. This method was chosen for this study because it requires a minimal, convenient and quick preparation of materials [16]. The characteristics of UV spectra samples were classified in the wavelength range of 210-400 nm (Figure 2). The results of the UV spectra (210-400 nm) of the infusion samples of akway bark, cinnamon, and the mixture of akway bark and cinnamon are presented in Figure 2.

The three types of solutions showed similar spectra with peaks around 278, 286, and between 285-290 nm for akway bark, cinnamon bark, and the mixture of both, consecutively. However, the three profiles of spectra were not yet distinguishable from each other thus further application of chemometrics techniques was required [17]. According to the previous study, the capability of the development of a spectral predictive model can be linked with the UV spectrum profiles, allowing researchers to observe metabolites from plants effectively [18].

3.1. Exploratory data analysis

Principal Component Analysis (PCA) is a data processing technique for developing linear multivariate models from complex data sets. PCA allows visualization of data groupings and pre-evaluation of similarities between groups or classes through the correlation by considering chemical or chemical-physical properties [19]. Data processing from the analytical instrument using UV spectroscopy in the range 210-400 nm was conducted to generate a PCA model (Figure 3). From this wavelength range, a selection was made to determine the contributing wavelengths in each sample. Since there were profiles of spectral slope at the range of 245-255 nm and profiles of spectral curve peak at the range of 275-299 nm, a total of 36 distinctive wavelengths were identified in each sample. They are 245, 246, 247, 248,

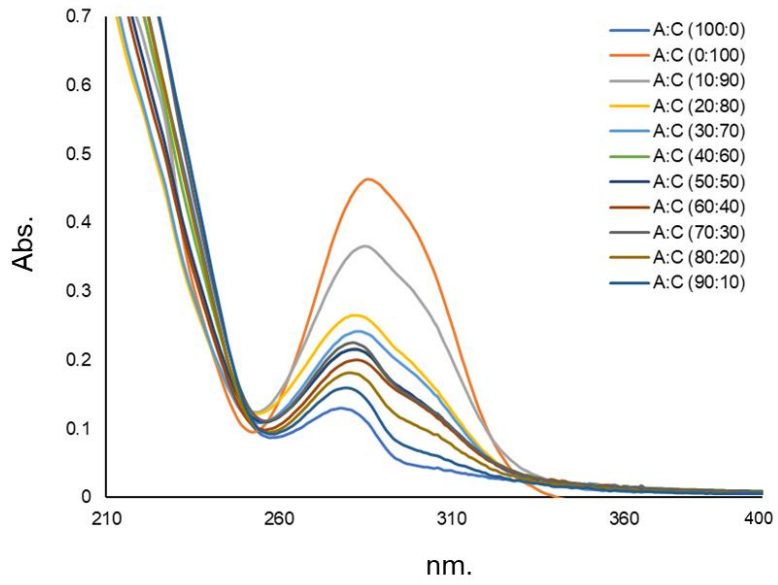


Figure 2. UV spectra of the solution of akway tree bark, cinnamon tree bark, and a mixture solution containing akway and cinnamon tree bark. The number in the brackets indicate the mixing ratios created to observe the patterns of (A) the akway tree bark solution and (C) the cinnamon tree bark solution

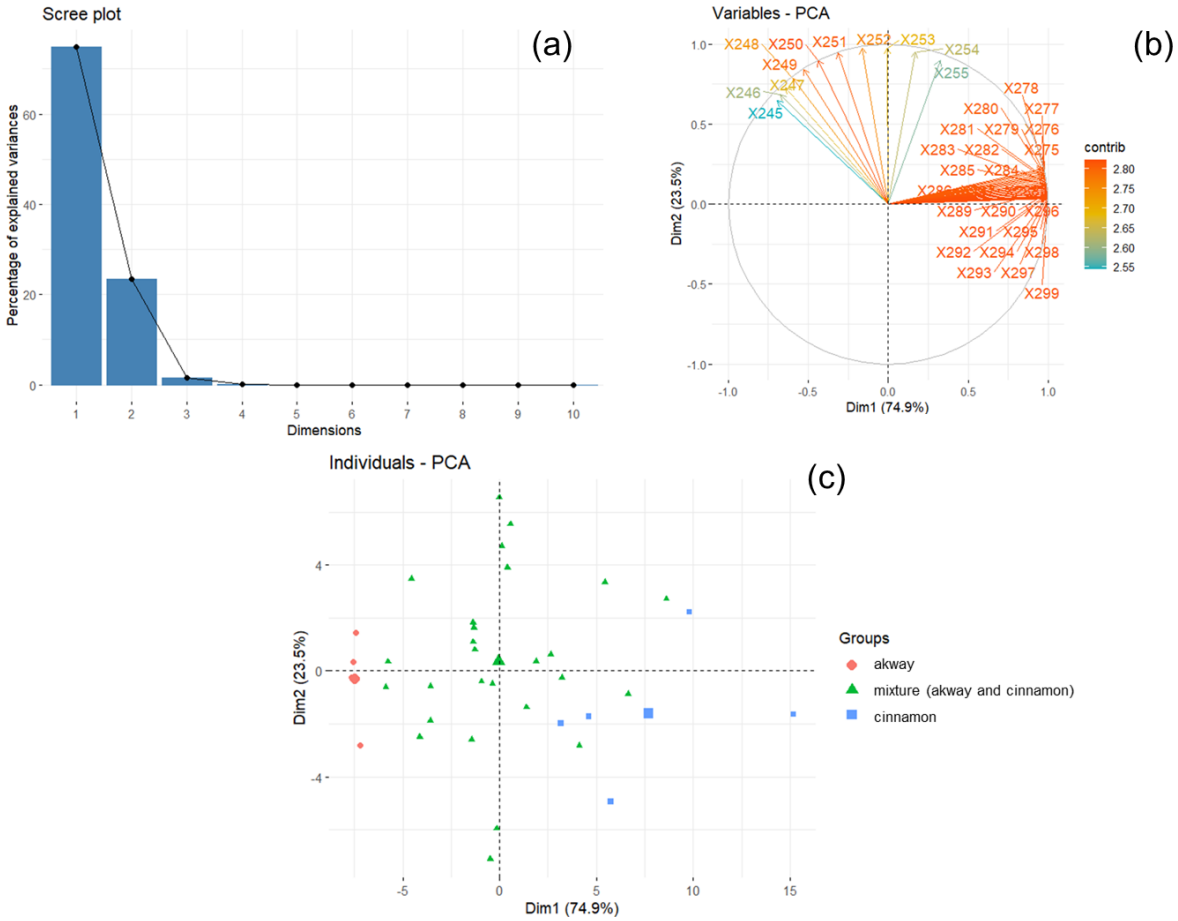


Figure 3. Results of principal component analysis of the original spectra including: (a) scree plot, (b) variable plot, and (c) individual plot

249, 250, 251, 252, 253, 254, 255, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, and 299 nm. This spectral identification approach has been applied in this study due to the possibility of generating a predictive model by assessing the pattern from UV-Vis spectra [20].

Based on the observation and evaluation of spectra, 36 wavelengths were selected as variables to form the PCA model, including 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, and 299 nm. The absorption values of each wavelength were used to form a PCA model. The results obtained were in the form of: (1) a scree plot used for visual assessment which showed the amount of variation possessed by each component or dimension, (2) a variable plot that was useful in evaluating the variables forming the main component through the angle between the vectors, and (3) an individual plot that represented the position of individual data on a two-dimensional display for two components [21]. Based on the scree plot, it was found that the involvement of two dimensions in the visualization indicated a total variance of 98.4% with the contributions from Dim1 (74.9%) and Dim2 (23.5%).

3.2. Multivariate calibration analysis

In this study, multivariate calibration was developed using PCR and PLS techniques [22]. These two techniques were used to predict the concen-

Table 2. The performance of principal component regression (PCR) and partial least squares (PLS) for predicting the content of akway tree bark and cinnamon tree bark in the infusion

Samples	Multivariate calibration	Type of spectra	n	R_{cal}^2	RMSEC	R_{CV}^2	RMSECV	R_{val}^2	RMSEP
Akway	PCR	**Original**	**3**	**0.933**	**6.709**	**0.910**	**7.748**	**0.958**	**5.292**
		First derivative	4	0.922	7.199	0.891	8.520	0.963	4.990
		Second derivative	16	0.999	0.379	0.640	15.490	0.825	10.806
		SNV	2	0.926	7.012	0.907	7.869	0.967	4.666
		SG	3	0.932	6.753	0.903	8.037	0.955	5.451
	PLS	Original	3	0.933	6.679	0.904	8.013	0.957	5.349
		First derivative	2	0.910	7.744	0.881	8.917	0.934	6.694
		Second derivative	4	0.999	0.687	0.644	15.420	0.831	10.628
		SNV	2	0.928	6.929	0.903	8.044	0.971	4.399
		SG	3	0.933	6.703	0.900	8.152	0.956	5.440
Cinnamon	PCR	**Original**	**3**	**0.933**	**6.709**	**0.910**	**7.748**	**0.958**	**5.292**
		First derivative	4	0.922	7.199	0.891	8.520	0.963	4.990
		Second derivative	16	0.999	0.379	0.640	15.490	0.825	10.806
		SNV	2	0.926	7.012	0.907	7.869	0.967	4.666
		SG	3	0.932	6.753	0.903	8.037	0.955	5.451
	PLS	Original	3	0.933	6.679	0.904	8.013	0.957	5.349
		First derivative	2	0.910	7.744	0.881	8.917	0.933	6.694
		Second derivative	4	0.999	0.687	0.644	15.420	0.831	10.628
		SNV	2	0.928	6.929	0.903	8.044	0.971	4.399
		SG	3	0.933	6.703	0.900	8.152	0.956	5.440

Note: Selected models of calibration for each compound were marked with bold. PCR: Principal Component Regression; PLS: Partial Least Squares; SNV: Standard NormalVariate; SG: Savitzky-Golay smoothing with polynomial order of 3 and window width of 11 points; n: number of components.

tration percentage of akway bark and cinnamon within the infusion to obtain the best prediction model in various modes of spectra (Table 2). The absorption data in the range of 210-400 nm were further processed through the pre-processing stage to produce original, first derivative, second derivative, standard normal variant (SNV), and Savitzky-Golay (SG) spectra. The prediction plot of the concentration percentage of akway bark and cinnamon in the infusion made from the best calibration model is presented in Figure 4.

The production of multivariate calibration models using PCR and PLS has been successfully conducted. The pre-processing stage enabled various types of spectra to be obtained including original, first derivative, second derivative, SNV, and SG spectra [23]. The overall output parameters were then evaluated, where a good R^2 value is one that is close to 1, while a good RMSE is one that shows the smallest value [24]. Evaluation was conducted on calibration, validation, and cross-validation data using the leave-one-out technique [25]. Based on the model performance evaluation results, it was found that PCR on the original spectrum showed the best quality of forming a multivariate calibration model for both akway bark and cinnamon bark infusions.

3.3. Discrimination using sPLS-DA

Spares partial least squares discriminant analysis (sPLS-DA) is an extension of sparse partial least squares (sPLS) regression that was applied for classification and discrimination purposes [26]. sPLS-DA was successfully produced for the original spectrum along with graphical visualization (Figure 5) and it displayed a more effective and informative data presentation in the authentication of akway bark infusion.

The sPLS-DA model on the background prediction plot provided visualization and discrimination markers for each group. The classification of sample categorization was developed using a maximum likelihood model. For samples of akway bark, cinnamon, and a mixture containing both, the AUC-ROC plot illustrated the ability of the sPLS-DA model to discriminate. The AUC curve indicated the degree of separation of the model, while the ROC curve represented the probability of discrimination of the model [15]. There were 3 classes: akway bark showed 1 or 100%, cinnamon showed 0.956 or 95.6% while the mixture showed 0.633 or 63.3%. Based on these results, it was found that the sPLS-DA technique successfully discriminated the akway bark infusion against the adulterant in the form of cinnamon bark infusion by using UV spectroscopy and chemometrics techniques.

4. Conclusions

This study developed a rapid detection technique of akway bark to prevent it from adulteration using UV spectroscopy method combined with chemometrics techniques. Exploratory data analysis was conducted using the PCA technique, followed by developing a multivariate calibration model using the PLS and PCR and conducting dis-

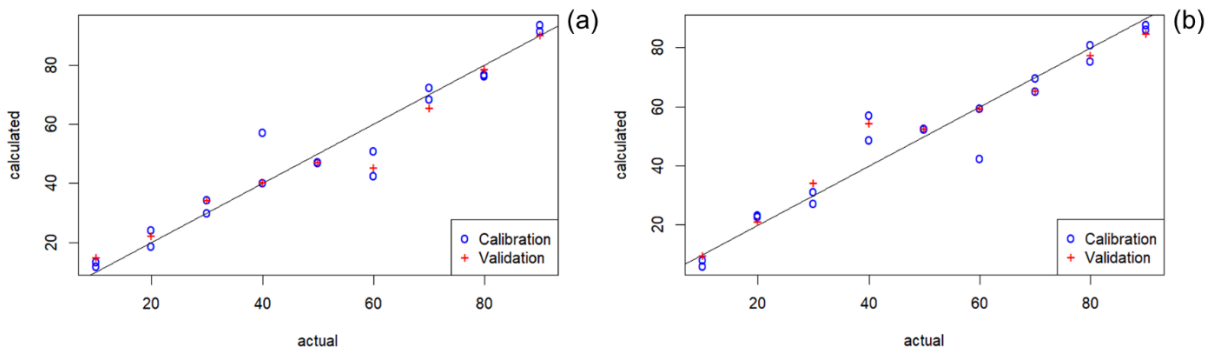


Figure 4. Prediction plots of (a) akway tree bark infusion and (b) cinnamon tree bark infusion generated from the selected calibration model for each sample

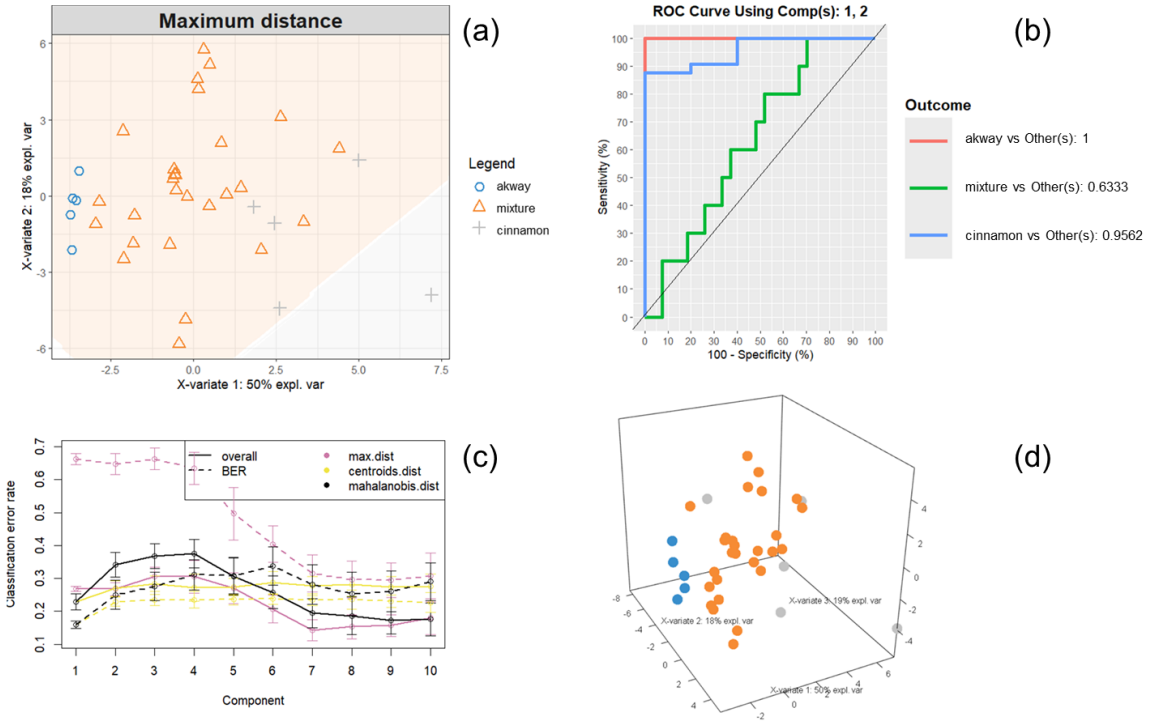


Figure 5. Output of sPLS-DA processing for akway tree bark infusion authentication, including (a) background prediction plot, (b) AUC-ROC plot, (c) classification error rate plot, and (d) 3D individual plot

crimination using sPLS-DA. The best multivariate calibration model was obtained from the original spectrum which was processed using PCR for both akway bark and cinnamon bark. The sPLS-DA model was successfully produced and discriminated with the AUC-ROC values of 1.000, 0.956, and 0.633 for akway bark, cinnamon, and a mixture containing akway bark and cinnamon, respectively.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

Authors would like to acknowledge the local community in Arfak Mountains, Manokwari, Papua, Indonesia, for the assistance in sample collection. Authors also thank to Sanata Dharma University, Indonesia for providing laboratory infrastructure to conduct this study.

Limitations of the study

This study has potential limitations. We acknowledge that taxonomic identification is crucial to be included in this research. However, we were unable to obtain the identification documents due to limited access, infrastructure, and documentation in the rural and restricted areas. To confirm the species identity, we consulted with a local community leader and conducted the identification at a local laboratory in Papua. Documentation of the *Drimys piperita* Hook f. tree bark used in this study is provided as Figure 1.

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