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Authentication Of Combretum Indicum Varr. B Flower Against Varr. M With Combined Chemometrics Of Uv-Vis Spectrophotometric

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A R T I C L E I N F O

Keywords:

Authentication
Melati belanda,
UV-VIS spectroscopy

ABSTRACT

Melati belanda flowers have two varieties that look different, namely from the shape of the flowers. The first variety has the shape of an elongated flower and the second variety has the shape of a rounded flower. So that, this difference in the shape of the flower will allow generating different activities. The purpose of this study was to identify the distinctive spectra of these two varieties. The method used is a fingerprint analysis of UV-VIS spectroscopy combined with chemometrics for the identification and authentication of varieties of Melati belanda. The results of the analysis of PLSR Combretum indicum Varr. B against Combretum indicum Varr. M on a spectrum of 218.86-252.54 nm, The first derivatization of RMSEC 2.39; R2 0.9975; RMSEP 6.92; R2 0.9939 and RMSEC 5.63; R2 0.9868. Meanwhile, with a wavelength of 253,260 – 299,020, a normal model of RMSEC 2.01 was obtained; R2 0.9983; RMSEP 1.89; R2 0.9985 and RMSEC 1; R2 0.9772. So can be concluded below UV-VIS spectroscopic fingerprint analysis combined with chemometrics is able to identify the authentication of the Combretum Indicum Varr. B against the occurrence of adulteration of flowers Combretum Indicum Varr. M at a wavelength of 253.260 – 299.020 nm of normal models.

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1. Introduction

Indonesia is one of the countries that has a very high biodiversity. Most of the many plants that can be used as traditional medicine, namely around 20.000-30.000 types that have the potential to be used for the needs of the pharmaceutical industry (Dogomo et al., 2020). One of the plants that has been used by the community is Melati belanda with a latin name (Combretum Indicum). The plant is used as a folk remedy such as, anthelmintic, anti-pain, diarrhea remedy, headache, rheumatism, immunomodulatory, antioxidant, anti-staphylococous and anti-inflammatory (Astuti et al., 2017). The Melati belanda plant has several properties, namely in fruit decoction it can be used for mouthwash, the leaves can be used to relieve pain due to fever, the flowers are used to relieve headaches, the seeds are used for deworming (Sahu et al., 2012). The Melati belanda plant can also be used to treat vaginal discharge, diabetes, colds, and ordinary coughs (Bahuguna et al., 2016) .

The chemical content of the Melati belanda plant in general contains potassium quisqualate. Its leaves and flowers contain tannins, glycosides, phenols, flavonoids and terpenoids. Melati belanda flower extract contains essential oils, E-and Z-linalool oxide (furanoid form), benzyl benzoate, 2,2,6-trimethyl-6- vinyl-3hydroxy tetrahydropyran (linalool oxide pyranoid form(Zuraida et al., 2017). The leaves contain rutin, trigonelin, L-proline, laspargin and quisqualic acid. Seeds contain linoleic, oleic, palmitic, stearic and arakidic acids. ellagitannins, quisqualin A and quisqualin. Fruits contain substances sugars similar to levulose and organic acids similar to cathartic acids(Shah et al., 2019) . The research that has been carried out on Melati belanda is used as an antihyperlipidemia, antipyretic, antibacterial, immunomodulatory and antioxidant(Valeri et

al., 2015). Other studies have shown that Melati belanda flower extract has significant antioxidant activity, namely in the activity of the DPPH radical antidote obtained an IC50 value of 40.92 µg / mL, in the method of superoxide radical antidote activity obtained an IC50 value of 38.76 µg / mL and in the radical cation-fighting activity (ABTS +) an IC50 value of 54.22 µg / mL. However, this Melati belanda has two varieties that look different are the shape of the flower, the first variety has elongated flower shape and the second variety has a rounded flower. The different shape of this flower will allow generating different activities. So, the purpose of this study is to identify the distinctive spectra of these two varieties with an authentication and adulteration approach.

Identification and authentication of plant species in 1 genus or 1 species that are part of the quality control of herbal medicine can be done by the fingerprint method of chemical compounds. Some analytical techniques such as thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), UV-VIS spectrophotometry, infrared, core magnetic resonance (CMR), mass spectroscopy (MS) or a combination of chromatography and mass such as GC-MS or HPLC-MS can be used for fingerprint analysis(Liang et al., 2004).

Until now, there have been no publications related to the use of UV-VIS spectroscopy fingerprints combined with chemometrics for combretum indicum flowers for identification and authentication purposes. Therefore, this study developed a UV-VIS spectroscopy fingerprint analysis method combined with chemometrics for the identification, discrimination, and authentication of varieties from Melati belanda. The method used in the authentication of Combretum Indicum Varr flowers. B against Combretum Indicum Varr. M is UV-VIS spectrophotometry with partial least square approach

2. Method

2.1 Materials:

Combretum indicum Varr. B flower (6 petals), Combretum indicum Varr. M flower (5 petals) 96% technical ethanol, and methanol p.a.

2.2 Instrumentation:

Micropipette 10-100 µl; micropipette 100-1000 µl. glassware (Pyrex and Iwaki), aluminum foil, 40 mesh sieve, maserator vessel, blender (Phillips), stirring rod, porcelain cup, chamber, glass funnel, filter paper, analytical balance (Ohauss, Pioneer), oven (Finco In OV 50), measuring flask, ruler, tweezers, capillary pipe, measuring pipette, test tube rack, UV-Vis spectrophotometry (Perkin Elmer Lambda 365), test tube and waterbath (Menmert).

Procedure

2.3 Sample Preparation

Fresh leaves are washed with clean water, drained, cut into small pieces, and dried in an oven of 50oC. After that, the dried leaves are ground and sifted using a 40 mesh sieving to obtain a powdery shape.

2.4 Sample Preparation for Fingerprint Manufacturing UV-VIS Spectroscopy

Weighed as many as 50 grams of powder samples (elongated type and rounded type Melati belanda leaves) as shown in table 2. then extracted with 5 liters of ethanol. The sample extract is filtered with a filter. The results of the extract were prepared with methanol p.a to be read spectra using UV-VIS spectroscopy.

Table 1

Composition used in %

Combretum Indicum varr. B	Combretum Indicum varr. M
0	100
20	80
40	60
60	40
80	20
100	0

2.5 UV-VIS Spectroscopy Equipment and Conditioning

Optimizing fingerprint spectra from UV-VIS spectroscopy was obtained by scanning the spectra from a wavelength of 200 nm to 400 nm and scanning the spectra to obtain a spectral area which is the distinguishing characteristic of the two varieties of Melati belanda. A total of 50 mg of

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the sample from table 1. was dissolved with 50 mL of methanol p.a and the spectra annotation process was carried out.

2.6 UV-VIS Spectroscopy Analysis

Spectroscopic analysis works with multicolored (polychromatic) light beams from xenon lamps. The xenon lamp enters the monochromator and is converted to monochromatic light. Next, the monochromatic light is split into two rays. One part of the solution can be sampled and the other can be a standard or reference solution. The light beam that has passed through the sample and reference is passed to the detector. The transmittance data (T) is obtained by comparing the value of the transmitted light intensity from the sample and the reference and converting it into an absorbance value (A). The absorbance data from the UV-VIS spectrophotometer stored on a flash disk was transferred to excel and then processed by the chemometric method.

2.7 Chemometric Analysis

Chemometrics in its application uses multivariate data analysis to process data with many variables. Chemometric Analysis using PLS (Partial Least Square) using chemometric. Analyze this by looking at the RMSEC, RMSEP and RMSECV Values.

3. Results and Discussion

Each plant has specifications or substances or components that are different (unique). Authentic plants are plants that are free from adulteration, especially with regard to the composition, nature and purity of varieties, geographical origin, and manufacturing methods technology. Plants are considered original if the content meets the specifications and information of existing standardization, to prove the authenticity of a plant can be used the uniqueness of existing substances or components such as physical properties, chemistry, DNA, and so on. In its development, there is also an authentication process that can be carried out with the help of laboratory tests such as UV-VIS spectrophotometry, FTIR HPLC or TLC.

Chemometrics is a science that obtains data by practicing mathematical and statistical methods. This technique is used to collect and analyze protocol multivariate data, calibrate, model processes, recognize patterns and classifications, signal correction and compression, and control statistical processes. Chemometric technique as a good solution for analyzing mixed compounds that have overlapping spectra profiles. The advantages of quantitative chemometric analysis techniques are that mixed compounds can be analyzed without a separation procedure, the technique is very easy to apply, very sensitive, useful, and very cheap compared to other analyzes (Putri et al., 2019; Serva et al., 2021).

UV-VIS spectroscopy is an analysis technique that uses a source of ultraviolet electromagnetic radiation and visible light using a spectrophotometer instrument. The principle of the UV-VIS spectrophotometer is an absorption of visible light for ultraviolet with a molecule that can result in the excitation of molecules from low energy levels to higher energy levels (Saputra Harahap et al., 2020). Dual beam spectrometers have various important components including light sources made of xenon lamps, monochromators, beam splitters, detectors made of dual silicon photodiodes, and as many as 6 sampel holders (Dinata, 2019).

3.1 Authentication *Combretum Indicum* Varr. B against *Combretum Indicum* Varr. M

Data obtained from the profile *Combretum Indicum* Varr. B, and *Combretum Indicum* Varr. M and sample mixtures were evaluated using multivariate chemometric analysis. Authentication is done by grouping % of the mixture and absorbance. The model was validated with RMSE (Root Mean Square Equation), RMSEP (Root Mean Square Equation Prediction), and RMSEV (Root Mean Square Equation Validation). Authentication is a process carried out to find out the existence of errors, forgeries or errors in determining raw materials in the form of plants that have the potential to be medicinal, because of their similarity in morphology and from their chemical content between varieties and even species(Subositi et al., 2016).

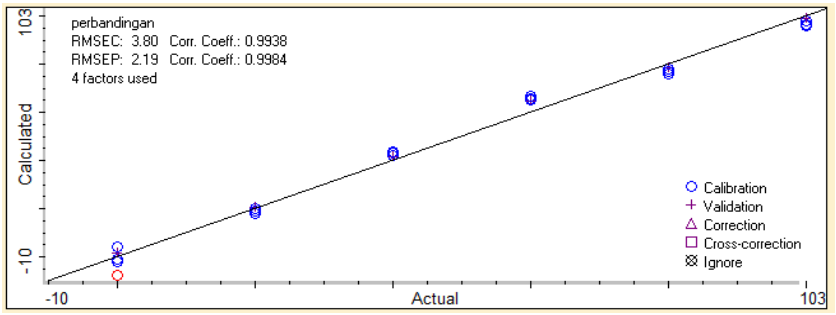

Figure 1. Authentication *Combretum Indicum* Varr. B against *Combretum Indicum* Varr. M at wavelengths 253,260 - 299,020 (normal model)

Figure 1 shows the relationship between the actual value and the predicted value obtained based on a combination of UV-Vis and chemometric spectroscopy. The wavelength used is 218.86 nm to 252.54, and the performance of the index is 92.

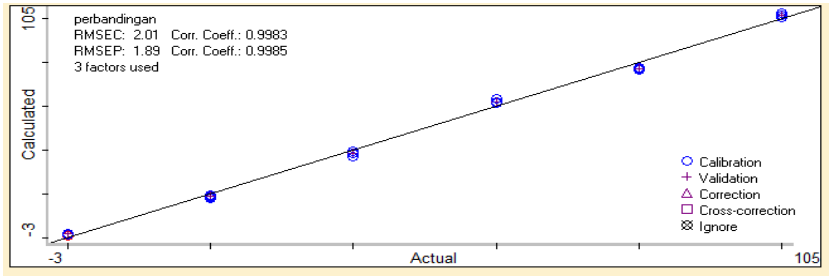

Figure 2. Authentication *Combretum Indicum* Varr. B against *Combretum Indicum* Varr. M at a wavelength of 253,260 - 299,020 nm (normal model)

The wavelength used according to what is shown in figure 2 is 253,260 to 299,020, the performance is 93.1. Figure 1 shows the relationship between the x-axis (actual concentration) and the y-axis (predictive concentration) obtained from a combination of UV-VIS spectroscopy and chemometrics. The results obtained can be seen based on the difference in RMSEC and RMSEP values to determine calibration with the PLS model (Prayitno *et al*., 2021). The accuracy of the model can be seen in the correlation value or coefficient of determination and the resulting error value(Rumoroy et al., 2019). The RMSEC value is used to determine the calibration model error. The smaller the RMSEC value, the smaller the error or error of the calibration process, so the more valid the method will be. The next parameter is RMSEP. A very small RMSEP value indicates that this modeling has good predictive ability (Guntarti *et al*., 2020).

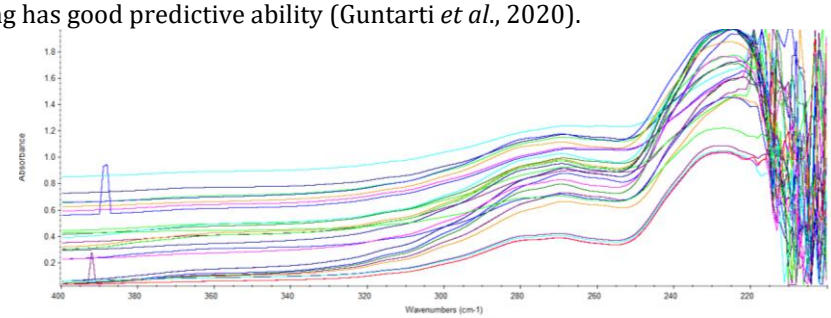

Figure 3. Spectra *Combretum Indicum* Varr. B and *Combretum Indicum* Varr. M

Figure 3 shows the spectra pattern of the entire existing data so that authentication is needed as a method to prevent sample forgery(Putri et al., 2019). Authentication can be performed using a spectroscopic UV-Vis tool. UV-VIS spectroscopy has the advantage of being affordable and widely available in the laboratory(Yulia et al., 2021). The wavelength range aims to maximize in the

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authentication process because the uptake of mixed samples will be quite significant(Rohaeti et al., 2019). The ultraviolet spectrum pattern appears to be a rounded type Melati belanda extract (*Combretum indicum* Varr. B) and elongated type Melati belanda (*Combretum indicum* Varr. M) on figure 3. shows maximum uptake in the wavelength range of 218.86-252.54 nm and 253.260-299.020 nm. The two wavelength regions are expected to transition $\pi \rightarrow \pi^*$ (conjugated C=C) from methanol extract solutions.

Table 2.
Analysis data of partial least square regression Combretum Indicum Varr. B and Combretum Indicum Varr. M spectra range 218.86 - 252.54 nm

type	performance index	calibrate		predict		cross-validation	
		RMSEC	R^2	RMSEP	R^2	RMSECV	R^2
Usual	92	3.8	0.9938	2.19	0.9984	9.59	0.9681
1 st	**74.9**	**2.39**	**0.9975**	**6.92**	**0.9939**	**5.63**	**0.9868**
2 st	-8.7	30.2	0.4697	29.9	0.5023	36.9	0.1457

PLSR multivariate calibration technique is often used for complex mixed analysis, as it determines each component in the mixture in a short period of time. The parameters of the PLSR multivariate calibration technique can be seen in the values of R2 (*square*) and RMSEC (Root Mean Square Error Calibration). The value of R2 is the linearity between the predictor variables against the response variable. The higher the coefficient value of determination (R2) and the lower the error value (RMSEC) indicates the better the calibration model (Rohaeti et al., 2019). The results of the analysis of PLSR *Combretum indicum* Varr. B and *Combretum indicum* Varr. M on the spectrum of 218.86-252.54 nm can be seen in table 2, that is, the first derivative is obtained is the best calibration model with a calibration R2 value of 0.9975 and an RMSEC value obtained of 2.39.

Table 3.
Analysis data of partial least square regretion *Combretum Indicum* Varr. B and *Combretum Indicum* Varr. M spectra range 253,260 - 299,020 nm

type	performance index	calibrate		predict		cross-validation	
		RMSEC	R^2	RMSEP	R^2	RMSECV	R^2
Usual	**93.1**	**2.01**	**0.9983**	**1.89**	**0.9985**	**1. 0000**	**0.9772**
1 st	84.5	4.3	0.992	4.26	0.9922	9.32	0.9634
2 st	81.5	2.67	0.9969	5.1	0.991	9.46	0.9638

Analysis data of partial least square regretion *Combretum Indicum* Varr. B and *Combretum Indicum* Varr. M spectra range 253,260 - 299,020 nm uses three models are normal model, first derivative, and second derivative. Each model has different performance index, R2, RMSEC, RMSEP, and RMSECV values. The value of R2 can be obtained using PLS calibration. The RMSEP value will indicate whether the calibration and validation model used is accurate and precise (Putri et al., 2019). The results of the analysis showed that from three model data analysis of partial least square regretion *Combretum Indicum* Varr. B and *Combretum Indicum* Varr. M obtained the best value on normal models with a calibration R2 value of 0.9983 and RMSECV value of 1.0.

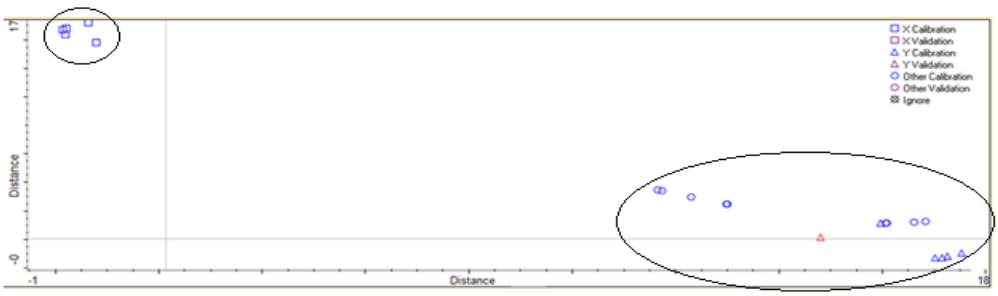

Figure 4. Coomaan plot between *Combretum indicum* Varr. B gainst mixing *combretum indicum* Varr. M

Figure 4. shows the plot score of the discriminant model. The score plot shows how the proximity between samples will show the discrimination of the sample into several groups(Yulia et al., 2017). The results of the plot showed a considerable distance between the *Combretum indicum* Varr B against the mixing group of *Combretum indicum* Varr. M and indicates an accuracy value of 100%, since none of the samples are incorrectly grouped.

4. Conclusion

So, it can be concluded under the analysis of UV-VIS spectroscopic fingerprints combined with chemometrics is able to identify the authentication of the *Combretum Indicum* Varr. B against the occurrence of adulteration of *Combretum Indicum* Varr. M at a wavelength of 253,260 - 299,020 nm of normal models.

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