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MINI REVIEW

Review on the application of chemometrics for the standardization and authentication of *Curcuma xanthorrhiza*

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Abstract

Temulawak (*Curcuma xanthorrhiza* Roxb.) or Javanese turmeric is one of Indonesia's native medicinal plants and is widely distributed throughout Southeast Asia. Temulawak contains some bioactive compounds having biological activities. The secondary metabolites in temulawak vary widely, depending on the environmental conditions where it grows. Temulawak as a raw material for herbal medicine is often faked with other rhizomes so that the analytical method capable of detecting the adulteration practice of temulawak is needed. The standardization of temulawak is a difficult task because the chemical compounds in temulawak are rather complex. In order to overcome the large and complex data, chemometrics is needed. The purpose of this paper was to highlight the application of chemometrics used during the standardization of temulawak through fingerprinting profile studies. During the literature searching, several databases namely Scopus, Web of Science, Pubmed and Google scholar were explored to get the relevant articles using specific keywords related to the topic. Some chemometrics techniques in combination with several instrumental techniques like spectroscopic and chromatographic methods are successfully used for the characterization and fingerprinting profiling of temulawak. Based on the data synthesized, chemometrics is powerful technique for treating the complex data intended for standardization of temulawak.

1. Introduction

In order to increase the preventive and promotive efforts in the health sector, herbal medicine is one of the people's choices to protect and maintain their health. Temulawak (*Curcuma xanthorrhiza*) is a native Indonesian medicinal plant widely spread to Southeast Asia (Nihayati *et al*., 2013). Temulawak, known as Javanese turmeric, is a rhizome widely applied as raw material for herbal and food ingredients. The demand for the herbal medicine industry is relatively high with an increase of 5.4% per year. The main chemical contents of *C. xanthorhiza* are xanthorrhizol accounting of 1.48–1.63%, curcuminoids such as curcumin, demethoxycurcumin, bisdemethoxycurcumin accounting of 1-2%, phelandren, camphor, tumerol, cineol, borneol, flavonoids, and sesquiterpenes (Husni, 2016). *C. xanthorrhiza* also contains some essential oils with chemical compounds of β-elemene, zingiberene, γ-elemene, β-farnesene, α-curcumene, benzofuran, α-cedrene, epicurzerenone, ar-curcumene, germacrone, aromadendrene, α longipene, trans-caryophilene and curcuphenol (Rafi, Septaningsih and Heryanto, 2018). These compounds are responsible for the yellow to orange color, as well as for the biological activities of temulawak (Itokawa *et al*., 1985).

Currently, the awareness and public concern in the standardization, authenticity, and quality of herbal medicines has increased significantly, therefore, analytical methods have been developed to perform these tasks (Rohman, Rawar, Sudevi *et al*., 2020). Herbal medicines have many complex chemical contents characterized by specific markers to differentiate plant species. Their biological activities are the cumulative effects of many chemical compounds (Jia *et al*., 2017). Some factors contribute to the biological activities such as time harvesting, seasons, plant age, therefore, some

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efforts are needed to standardize the herbal raw materials in order to ensure the quality of the herbs (Gopi *et al*., 2019). Medicinal plants with complex chemical contents, of course, require a special method for quality controls through physico-chemical and molecular biology analyses (Ni *et al*., 2009). The process of identification and analysis of chemical constituents in medicinal plants can be performed by three approaches namely single component analysis through analysis of specific markers, fingerprinting analysis and metabolomic (Esteki *et al*., 2018) using some instrumental techniques including molecular spectroscopic and chromatography methods (Mazina *et al*., 2015). Temulawak is widely applied in herbal and traditional medicine products. Due to its high demand, temulawak is the potential to be substituted or adulterated with other species having a similar appearance such as *Curcuma domestica* (Muttaqin, 2018), therefore, it is very important to standardize temulawak to assure its quality (Windarsih *et al*., 2021).

The standardization of herbal medicine using fingerprinting profiling and metabolomics resulted in large numbers of responses which make it difficult to handle them. Fortunately, the special statistical package known as chemometrics could resolve this problem. Almost fingerprinting profiling and metabolomic studies used chemometrics for special purposes including pattern recognition and multivariate calibration (Granato *et al*., 2018). Chemometrics is a combination of mathematical and statistical techniques to process chemical data (Rohman., 2017). Some reviews on the application of chemometrics in herbal standardization include Traditional Chinese Medicines (Razmovski-Naumovski *et al*., 2010; Bansal *et al*., 2014; Li *et al*., 2020). Therefore, the purpose of this paper is to highlight the application of chemometrics used during the standardization of temulawak. In authentication of herbal medicine, the identification of the geographical origin of a medicinal plant including *C. xanthorrhiza* is part of drug analysis and part of quality control in pharmaceutical analysis. Identifying the geographic origin of plant material is a difficult task to do chemically, thus, an application of chemometrics is required to perform these tasks (Xie *et al*., 2006).

2. Methods

During performing this narrative review, we followed some steps as suggested in several papers reporting the writing of the review articles (Green *et al*., 2006; Gasparyan *et al*., 2011; Gregory and Dennis, 2018). The databases used during searching works of literature needed for writing review articles were Web of Science, Scopus, PubMed and Google Scholar. The keywords used for information search are *Curcuma xanthorrhiza* + chemometric OR *Curcuma xanthorrhiza* + standardization OR *Curcuma xanthorrhiza* + geographic origin.

3. Chemometrics

According to the International Chemometrics Society (ICS), the definition of chemometrics is described as "the science of relating chemical measurements made on a chemical system to the property of interest (such as concentration) through the application of mathematical or statistical methods (Rohman and Windarsih., 2020). Chemometrics is a branch of science, which relates the chemical analysis, mathematical or statistical methods (Gemperline., 2006). In chemometrics, some analytical purposes namely quantitative analysis using multivariate calibration, identification or classification using supervised or unsupervised pattern recognition are commonly applied in chemical sciences including authentication and standardization of herbal medicines (Brereton, 2003; Rohman *et al*., 2014). For example, in pattern recognition using chromatography methods, the samples are grouped according to their measurement (responses) using chromatogram which is the specific character of the analyzed sample (Beebe *et al*., 1998). The variables used during this task can be retention time, peak area, and peak height. The data were displayed in the form of a matrix consisting of rows and columns written numerically. Each row relates to one object and each column relates to certain features of the object or samples (Massart *et al*., 1997). Chemometric methods in data analysis are pervasive and important in the decision-making and problem-solving processes. The chemical analysis deals with complex mixtures, compounds, and their properties, which are often very complicated to be analyzed. Therefore, chemometrics is suitable for the analysis of herbal medicines which are typically complex in nature (Rohman, Rawar, Sudevi *et al*., 2020).

Today with the sophisticated development of statistical software and computers, chemometrics have become the main tool for processing data (Bansal *et al*., 2014). Several chemometric techniques applied in the standardization and authentication of herbal medicine are data preprocessing such as normalization and derivatization, data exploratory using Principal Component Analysis (PCA), unsupervised pattern recognition using Soft Independent Modeling of Class Analogy (SIMCA) and cluster analysis, supervised pattern recognition such as discriminant analysis and multivariate calibrations such as partial least square regression and principle component regression (Bansal *et al*., 2014).

In herbal standardization including *C. xanthorrhiza*,

the chemometrics techniques are applied during fingerprint profiling and metabolomic studies (Bansal *et al*., 2014; Rohman, Ghazali, Windarsih *et al*., 2020). While in the authentication studies, chemometrics assisted in determining the origin of herbal medicine, adulteration of high-quality components of herbal medicines with the lower one, or identification of undeclared components in herbal products (Liu *et al*., 2020). Fingerprint profiling can be obtained from spectroscopic, chromatographic or electrophoretic data. The fingerprint profile must be able to display the similarities and differences in the analyzed samples (Cubero-Leon *et al*., 2014). The authentication of herbal medicine is a difficult task because many components of medicinal plants are unknown (Bauer, 1998). Fortunately, the chromatography and spectroscopy methods are able to show very strong authentication techniques through fingerprinting profiles. Combining the chemometrics with instrumental responses, the standardization of herbal medicine can be achieved with high precision and accuracy (Gad *et al*., 2013).

4. Application of chemometrics in standardization of *Curcuma xanthorrhiza*

Some chemometrics techniques of pattern recognition and multivariate calibrations combined with some instrumental techniques of spectroscopic and chromatographic methods were applied for the standardization of Temulawak. The variables used for chemometrics analysis using spectroscopic methods are absorbance values or ratios at specific wavelengths or wavenumbers, while variables exploited using chromatographic techniques were retention time, peak area, peak height or its ratios (Li *et al*., 2020). Table 1 compiled some chemometrics methods combined with instrumental techniques for the identification and discrimination of Temulawak, intended for standardization as described in Table 1.

4.1. Principal component analysis

Principal component analysis (PCA) is an exploratory data analysis commonly used for the classification of samples (Irnawati *et al*., 2021), and two outputs in PCA commonly reported during classification or clustering are principal components (PCs) and loading plots. PCs are useful to identify any groupings in the data set. In addition, loading plots are shown from coefficients by which the original variables are multiplied in order to get PCs values. Loading plots describe the variables responsible for the separation and or classification of objects (Kim *et al*., 2010). The identification and discrimination of similar plants, such as turmeric (*C. longa*), Javanese turmeric (*C. xanthorrhiza*) and Bangle (*Zingiber cassumunar*), need to be done to ensure the quality of raw materials used (Rohaeti *et al*., 2015). Fourier transform Infrared (FTIR) spectroscopy combined with chemometrics can be the method of choice because of the analysis due to its nature as a fingerprint. Visual discrimination of the three species is indicated by the marker bands of FTIR spectra of each species. PCA followed by Canonic Variate Analysis (CVA) using FTIR spectra could classify these plants (Rohaeti *et al*., 2015).

PCA and discriminant analysis in combination with UV spectroscopy have been applied for the differentiation of four Curcuma species, namely *Curcuma xanthorrhiza*, *C. longa*, *C. aeruginosa* and *C. mangga*. These four rhizomes are widely used in herbal medicine and dietary supplements. The absorbance values of UV-Vis spectra at the wavelength of 210-500 nm were used as variables during differentiation. UV-Vis spectra were acquired in the interval of 200-800 nm and the standard normal variate was used for preprocessing the spectral data. Using two PCs (PC1 and PC2), PCA could differentiate Curcumas in which PC1 and PC2 were accounting for 79.30% and 12.0%, respectively. In addition, DA using discriminant function 1 (DF1) and discriminant function 2 (DF2) could discriminate four species with an accuracy rate of the correctness of 95.5% (Rafi *et al*., 2018). PCA is also applied for the differentiation of *C. xanthorrhiza*, *C. aeruginosa*, and *C. longa* using two-dimensional NMR spectra. PC1 and PC2 were accounting for 63.1% and 28.1%, respectively. Based on the identification of metabolites, curcumin and xanthorrhizol are responsible for this differentiation (Wahyuni *et al*., 2019).

Multivariate analysis of PCA using data set obtained from ^{1}H-NMR spectra clearly discriminated pure and adulterated *C. xanthorrhiza* with *C. aeruginosa* as shown in Figure 1. PCA using two PCs showed a clear separation between pure *C. xanthorrhiza*, pure *C. aeruginosa*, and adulterated *C. xanthorrhiza* using several concentration levels of *C. aeruginosa*. Several original variables used for making the PCA model were reduced to be principal components, which explains the original variables. PC1 and PC2 described 73% of PC1 and 24% of PC2.

Research has been carried out on the existence of adulteration of *C. xanthorrhiza* with *C. domestica*, based on the fingerprint profiling by Thin Layer Chromatography (TLC). Fingerprinting profiles of *C. xanthorrhiza* were obtained from *C. xanthorrhiza* from Cianjur, Semarang, and East Nusa Tenggara, while the fingerprint profiles of *C. domestica* were obtained from Cianjur regions. Furthermore, the analysis was carried out by PCA. The results of PCA analysis showed that the

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Table 1. The application of several chemometric techniques for the identification and discrimination of *C. xanthorrhiza*, intended for standardization, authentication and quality controls

Chemometrics techniques	Methods used	Issues	Remark	References
PLSR	FTIR spectroscopy (4000-650 cm^{-1})	Standardization of *C. xanthorriza* by determining the contents of curcuminoid	The multivariate calibration of PLSR using absorbance at certain wavenumbers could predict the levels of curcuminoids in *C. xanthorriza* extract	Lestari *et al*. (2017)
PLSR	FTIR spectroscopy (4000-650 cm^{-1})	Quantitative analysis of curcuminoids in tablet	Curcuminoids in tablets containing *C. xanthorriza* extract could be predicted accurately	Siregar *et al*. (2018)
PCA, canonical variate analysis (CVA)	FTIR spectroscopy (4000–400 cm^{-1})	Differentiation of C. xanthorriza from other Curcuma species for authentication	These chemometrics using absorbance values at 4000-650 cm^{-1} as variables could discriminate Curcuma species for authentication issues of herbal medicine	Rohaeti *et al*. (2015)
PLSR and PCR	FTIR spectroscopy (4000-650 cm^{-1}) and HPLC	Determination of curcumin in *C. xanthorriza* extract for standardization	FTIR spectra and HPLC combined with this chemometrics could predict curcumin content for standardization studies	Rohman *et al*. (2015)
PCA	Uv-vis (DPPH at 515 nm)	Classification of *C. xanthorriza* based on antioxidant activities	PCA could classify *C. xanthorriza* extract based on geographical origin	Widyastuti *et al*. (2020)
PCA and PLSR	FTIR spectroscopy (4000 - 600 cm^{-1})	Differentiation and quantification of *C. xanthorriza* in Turmeric for authentication of turmeric	PCA could classify and differentiate Curcuma species, while PLS could predict the concentration of C. xanthorriza in Turmeric	Angeline *et al.* (2019)
PLS-DA	NMR spectroscopy	Authentication of *C. xanthorriza* from *Zingiber montanum* for authentication studies	Chemometrics of PLS-DA using 7 principal components (PCs) could classify between authentic and adulterated samples of *C. xanthorrhiza*	Windarsih *et al*. (2021)
PCA and OPLS-DA	NMR spectroscopy and TLC	Authentication of *C. xanthorriza* from *Curcuma aeruginosa* intended for authentication studies	1H-NMR-based metabolite fingerprinting coupled with PCA and OPLS-DA offers an adequate method to assess adulteration practice and to evaluate the authentication of *C. xanthorrhiza* extracts.	Rohman, Ghazali Windarsih *et al*. (2020)
Discriminant analysis	HPLC	Differentiation of *C. xanthorriza* from other Curcuma species for authentication studies	Discriminant analysis using variables of retention time and peak area could differentiate Curcuma species from different origins	Rafi *et al*. (2015)
PCA	NMR	Differentiation of Curcuma species including *C. xanthorriza* for authentication studies	Two-dimensional (2D)-NMR spectroscopy could differentiate Curcuma species based on metabolites contained in Curcuma species	Wahyuni *et al*. (2019)
PCA and Discriminant analysis (DA)	UV-vis spectroscopy (200-800 nm)	Differentiation of Curcuma species including *C. xanthorriza* for authentication studies	DA offered a better classification of *C. xanthorriza* with a success rate of 95.5%	Rafi *et al*. (2018)

fingerprints of *C. xanthorrhiza* and *C. domestica* were in the different quadrants. However, analysis of instant curcuma samples showed that the samples were in the quadrant between *C. xanthorrhiza* and *C. domestica* (Muttaqin *et al*., 2018). PCA was also successfully used for the classification of other Curcuma species intended for hindering the adulteration practice (Windarsih *et al*., 2019).

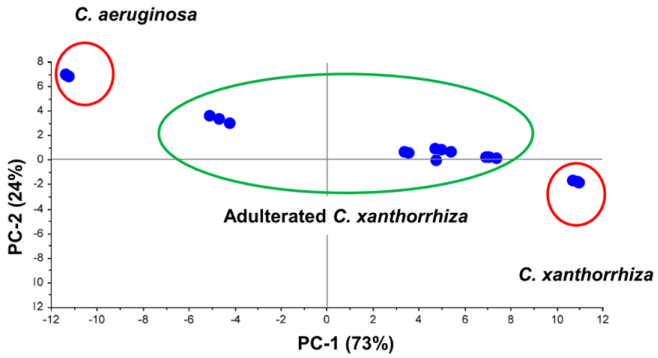

Figure 1. The score plots of principal component analysis for classification of pure *C. xanthorrhiza,* pure *C. aeruginosa,* and adulterated *C. xanthorrhiza*. The output was obtained using SIMCA 14.0 software (Sartorius, Malmo, Sweden). Source: Rohman, Wijayanti, Windarsih *et al*. (2020).

4.2 Discriminant analysis

Discriminant analysis (DA) is one of the supervised pattern recognitions commonly used for the discrimination or classification of objects/samples into several groups (Rohman and Putri, 2019). DA using algorithm of orthogonal projections to latent structures (OPLS) has been applied for the authentication of *C. xanthorrhiza* with *C. aeruginosa* using variables of ^{1}H-NMR spectra. OPLS-DA was successfully applied for the classification of pure and adulterated *C. xanthorrhiza* with higher R2X (0.965), R2Y (0.958), and Q2(cum) (0.93) as shown in Figure 1 (Rohman, Wijayanti, Windarsih *et al*., 2020). Authentication and discrimination studies have also been conducted to differentiate the fingerprints of *C. xanthorrhiza* and *C. longa* based on curcuminoid levels using HPLC assisted with chemometrics of DA. This combination can separate *C. xanthorrhiza* and *C. longa* species (Rafi *et al*., 2015). In addition, the combination of 1H-NMR and chemometric methods are promising for the authentication of medicinal plants (Windarsih *et al*., 2021). ^{1}H-NMR spectroscopy and chemometrics have been applied to authenticate *C. xanthorrhiza* adulterated with *Zingiber cassumunar*. Partial least square-discriminant analysis (PLS-DA) using 7 main components (PCs) was successfully classified the original sample and the adulterated *C. xanthorrhiza* with values of R2X (0.988), R2Y (0.998), and Q2 (0.993). The chemometrics of PCA and PLS-DA allows for the discrimination of pure *C. xanthorrhiza* and *C. xanthorrhiza* adulterated with *Z. cassumunar* (Wijayanti *et al*., 2019).

4.3 Multivariate calibrations

Multivariate calibration is one of the quantitative tools for prediction of analyte(s) of interest in herbal medicine like curcumin in Temulawak using several variables. Partial least square regression (PLSR) and principle component regression (PCR) are the most applied techniques (Keithley *et al.,* 2009). PLSR using absorbance values of FTIR spectra at 4000-650 cm^{-1} has been used to predict the levels of curcumin (C), demetoxycurcumin (DM) and total curcuminoid (TC) in *C. xanthorrhiza* intended for the standardization. The actual contents of curcuminoid in the ethanolic extract of *C. xanthorrhiza* were previously determined using HPLC with a PDA detector. With PLSR, the R^2 values of the calibration model for CUR, DMCUR and TCUR were > 0.99. The levels of curcuminoid determined using FTIR spectroscopy-PLSR were not statistically significant compared with the HPLC method based on an independent sample t-test ($P > 0.05$) (Lestari *et al*., 2017).

The combination of FTIR spectra-PLSR was also successfully applied for the prediction of the levels of curcumin in curcuma in *C. longa* and *C. xanthorhiza*. The actual levels of curcumin were determined using HPLC. PLSR using absorbance values at wavenumbers of 2000-950 cm^{-1} was suitable for the prediction of curcumin. The R^2 values for the correlation between actual values and FTIR predicted values of curcumin were 0.96 and 0.99 with RMSEC values of 0.299 and 0.089 in *C. longa* and *C. xanthorriza*, respectively. High R^2 values and low RMSEC values indicated high accuracy and precision of the analytical method (Rohman *et al*., 2015).

5. Conclusion

Several chemometrics techniques either pattern recognition (supervised such as discriminant analysis and unsupervised like principal component analysis) or multivariate calibrations like partial least square using variables generated from several instrumental techniques like spectroscopic and chromatographic methods are successfully used for characterization and fingerprinting profiling of herbal medicines including Temulawak intended for the authentication, quality control and standardization of herbal medicine. Based on the data synthesized, chemometrics is a powerful and meaningful technique for treating the complex data intended for the standardization and authentication of herbal medicines such as Javanese Turmeric.

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