

The use of chemometrics in combination with molecular spectroscopic and chromatographic methods for authentication of *Curcuma* species: a review

^{1,*}Rohman, A., ¹Rawar, E.A., ²Sri Sudevi, ³Nurrulhidayah, A.F. and ⁴Windarsih, A.

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada Yogyakarta 55281 Indonesia

²Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia

³International Institute for Halal Research and Training (INHART) Level 3, KICT Building, International Islamic University Malaysia (IIUM), 53100 Jalan Gombak, Selangor, Malaysia

⁴Research Division for Natural Product Technology (BPTBA), Indonesian Institute of Sciences (LIPI), Yogyakarta, Indonesia, 55861

Article history:

Received: 20 October 2019

Received in revised form: 2 January 2020

Accepted: 5 January 2020

Available Online: 30 June 2020

Keywords:

Molecular spectra,
Chromatography,
Chemometrics,
Curcuma longa,
Curcuma xanthorrhiza

DOI:

[https://doi.org/10.26656/fr.2017.4\(6\).345](https://doi.org/10.26656/fr.2017.4(6).345)

Abstract

Currently, the awareness and public concern in the authenticity of herbal medicines has increased significantly, therefore, analytical methods capable of detecting the adulteration practice must be available. The rhizomes of *Curcuma* species such as *Curcuma longa* and *Curcuma xanthorrhiza* are the target of adulteration due to its popularity as components in herbal medicine formulation. For the sake of quality control of herbal medicines, a rapid and reliable method must be developed for authentication studies. Molecular spectroscopy including UV-Vis, infrared (near and mid) and ¹H-NMR spectroscopy, as well as chromatographic-based methods especially liquid chromatography, can be an ideal method for herbal authentication due to its simplicity, however, the spectra and chromatogram obtained are usually complex which are difficult to interpret. To overcome this obstacle, a statistical approach known as chemometrics was used to treat spectra data to be easily used for authentication purposes including discrimination and classification between authentic and adulterated herbal components. This review highlighted molecular spectroscopic method in combination with multivariate data analysis (chemometrics) for authentication of herbal components.

1. Introduction

Curcuma species especially *Curcuma longa* and *Curcuma xanthorrhiza* are one of the herbaceous perennial plants of the ginger family which is native to tropical South Asia, but it is now widely cultivated in the subtropical and tropical regions. *C. longa* and *C. xanthorrhiza* is also widely cultivated in Indonesia and it has been put to use as a foodstuff, cosmetic, and medicine (Jurenka, 2009). *C. longa* extract has many chemical constituents. More than 100 components have been identified and the primary compounds are phenolic, terpenoids, diarylheptanoids (including curcuminoids), diarylpentanoids, and alkaloid (Li *et al.*, 2011). *C. xanthorrhiza*, also known as Javanese turmeric or *temulawak*, is widely used as traditional plant medicine in Indonesia and other regions. In Southeast Asia, Javanese turmeric is traditionally used as food essentials and medicine. In Indonesia, *C. xanthorrhiza* is not only utilized as home remedies such as *jamu* prescription,

food supplement, and herbal drink but also is extensively used as a raw material in pharmaceutical industries (Tajuddin *et al.* 2011; Ramdani, *et al.*, 2016).

C. longa and *C. xanthorrhiza* are known to have various pharmacological activities such as improving hepatic functions, promotion of blood circulation, anti-cancer, and anti-inflammatory activities (Cousins, *et al.*, 2007). The curcumin content in *C. longa* and *C. xanthorrhiza* assisted the recovery of liver function by reducing oxidative stress which damages normal cell (Farzaei *et al.*, 2018). The active curcuminoids on *C. longa* also showed hepatoprotective and cardioprotective effects (Mohanty *et al.*, 2004), hypoglycemic effect (Honda *et al.*, 2006), antioxidant (Cousins, *et al.*, 2007) and chemoresistance activities (Bar – Sela *et al.*, 2010). High amounts of curcuminoid and xanthorrhizol present in *C. xanthorrhiza* possess a variety of biological activities such as anticancer, antimicrobial, anti-inflammatory, antioxidant, anticandidal,

*Corresponding author.

Email: abdul_kimfar@ugm.ac.id

antihyperglycemic, and antihypertensive effects (Jantan *et al.*, 2012).

Because of its high quality and high demand for product containing Curcuma species, it is very important to ensure the authenticity of *C. longa* and *C. xanthorrhiza* to avoid adulteration or substitution with other plant species. It is also important to ensure that the product complies with its claims. Adulteration of medicinal plants is a common practice worldwide. Adulteration poses a serious problem because it is related to the safety, efficacy, and quality of the product. *C. longa* rhizome, especially the powder form is potential to be adulterated with other species of *Curcuma* (Remya, *et al.*, 2004). Adulterated products of *C. longa* and *C. xanthorrhiza* usually have a low content of curcuminoids. The common potential adulterant of *C. longa* and *C. xanthorrhiza* is *Curcuma manga*. Other *Curcuma* species such as *Curcuma heyneana* also may be used as an adulterant in *C. longa* and *C. xanthorrhiza* powder. *C. heyneana* and *C. manga* are potential to be used as adulterants because of their lower price, wide availability, and yellow color. Therefore, fast, reliable and reproducible analytical methods for the authentication of *C. longa* and *C. xanthorrhiza* are needed to be developed. One of the methods currently used for authentication of Curcuma species is molecular spectroscopy.

2. Methodology

During performing this review, we used several databases including Scopus, PubMed, and Google Scholar to identify and to download the abstracts, reports, and research papers related to authentication analysis of Curcuma species. The keywords used during searching of information was (authentication + *Curcuma longa* + spectroscopy + chromatography) and (authentication + *Curcuma xanthorrhiza* + spectroscopy + chromatography) in the month of June-August 2018.

3. Results and discussion

3.1 Molecular spectroscopy

Molecular spectroscopy is a field of study related to the interaction of electromagnetic radiation (EMR) in certain regions with samples in the molecular levels (to differentiate with atomic spectroscopy). UV-Vis spectroscopy is taken into account as an interaction between EMR in the region of 200-800 nm with evaluated samples, while infrared spectroscopy takes place in the region of 800-25000 nm (Rouessac and Rouessac, 2007; Skoog *et al.* 2007; Pavia *et al.*, 2009). Over the past two decades, there is an increasing interest

among communities of research and industry in the use of molecular spectroscopy methods which include ultraviolet-visible (UV-Vis), vibrational spectroscopy (infrared and Raman) and nuclear magnetic resonance (NMR). Molecular spectroscopies offer some advantages, namely non-destructive nature of the method, minimum or no sample preparation required, ease in operation and the speed of the analysis (Cozzolino, 2015). (UV-Vis spectroscopy can be used for qualitative and quantitative analyses based on the interaction of photon in the region of 200-400 nm (ultraviolet) and 400-800 (visible) with samples. This interaction resulted in an electronic transition of samples, in which electrons are excited from the ground state into an excited state. In order to quantify the analyte(s) of interest contained in Curcuma species, the absorbance based on Lambert-Beer law is exploited according to the equation of:

$$A = abc$$

Where A is absorbance or optical density; *a* = absorptivity or extinction coefficient; *b* is path length of radiation through sample (cm); and *c* is concentration of solute in solution (Pavia *et al.*, 2009)

The samples must have chromophores to be analysed with UV-Vis spectroscopy. The main components of *Curcuma* species are curcuminoid, refer to phenolics compounds of curcumin, demethoxycurcumin and bisdemethoxycurcumin having chromophoric groups, therefore, UV-Vis spectroscopy was suitable for authentication of Curcuma species (Rohman *et al.*, 2015).

Infrared (IR) spectroscopy, either in near or mid regions, have emerged as potential tools for analysis of pig derivatives. IR spectroscopy is fast, ease in instrument operation, minimum sample preparation or even without any sample preparation, non-destructive which means that samples measured using IR spectroscopy can be analysed using other instruments, and fingerprint in nature. However, the signal obtained is rather complex which make difficult to interpret, therefore, the use of chemometrics to solve the problems related to IR spectral interpretation and analysis is unavoidable (Rohman 2014; Wang *et al.*, 2016). IR spectroscopy is based on the interaction between samples with EMR in IR region resulted in vibrational transition of chemical bonds, therefore, IR along with Raman spectroscopy is called as vibrational spectroscopy (Rohman and Che Man, 2012). In IR spectroscopy, two units are frequently used namely wavelength (nm) and wavenumbers (cm⁻¹). IR regions are divided into three regions of near (0.8-2.6 μm or 14,000-400 cm⁻¹), mid (2.6-26 μm or 4000-400 cm⁻¹) and far (26-100 μm or 400

-10 cm^{-1}). To avoid large numbers and for convenience, the unit of wavelength (μm) is used during analysis with near IR and wavenumbers unit (cm^{-1}) is commonly used for analysis with mid-IR (Gendrin *et al.*, 2008). Mid-IR region is the most reported IR spectroscopies used for qualitative and quantitative analysis of pig derivatives due to large information obtained. The IR spectrum revealed wavenumbers in x-axis and intensity (transmittance or absorbance) in the y-axis, coming from vibrational and rotational transitions of chemical bonds among atoms within the molecule (Hannah, 2002). IR spectra are traditionally used for qualitative analysis (identification) by identifying specific functional groups present in molecules. In recent years, with the development of multivariate calibration, IR spectroscopy is widely exploited for quantitative analysis. For qualitative analysis, either absorbance or transmittance can be used. Meanwhile, the absorbance mode must be used in the quantitative analysis according to Lambert-Beer law (Griffiths and Chalmers, 1999).

Raman spectroscopy and IR spectroscopy are complementary methods. Raman spectrum is obtained by focusing monochromatic radiation on a sample and analyzing the scattered light as a function of frequency. This irradiation resulted in two types of light scattering, namely elastic and inelastic. Raman spectroscopy relies upon the inelastic scattering of EMR by a molecular system (Wartewig and Neubert, 2005). The elastic scattering occurring at the same wavelength as that of irradiated light is known as Rayleigh scattering. Due to gain or loss of energy, this inelastic scattering is accompanied by photon frequency shift and its wavelength is changed which results in Raman scattering. In Raman scattering, molecule subjected with an incident photon is excited from the ground state to the excited state, which immediately relaxed to ground electronic or vibrational states and undergo a wavelength shift (Kalantri *et al.*, 2010; Chen *et al.*, 2017). Raman spectra are presented as an intensity (y-axis) versus wavelength shift recorded over a range of $4000\text{--}10\text{ cm}^{-1}$ (y-axis). The spectrum of Raman is simpler than that of its counterpart of IR because bands due to Raman overtones, combination and difference are rare. Like IR spectrophotometer, Raman spectrophotometers can be dispersive or nondispersive using Fourier transformation (FT-Raman spectrophotometer) (Settle, 1997; Bumrah and Sharma, 2016).

Nuclear Magnetic Resonance (NMR) spectroscopy, based on the absorption of EMR by specific isotopes of atomic nuclei, has been extensively used for authentication analysis of food products and has been emerged as potential means for analysis of Curcuma species for authentication purposes (Dais and Hatzakis,

2013). Proton (^1H)-NMR spectroscopy is more preferred over ^{13}C -NMR due to its sensitivity and shorter relaxation time, make ^1H -NMR is faster analysis. NMR spectroscopy is taken into account as one of the most suitable methods to obtain "high throughput" spectroscopic methods containing information on the molecular chemical structure on large compounds. This method is capable of determining complex matrices with minimum sample preparation. NMR spectroscopy can analyze a large amount of metabolites including those appear in Curcuma species. Therefore, NMR spectroscopy especially in combination with chemometrics is ideal for metabolomics analysis for authentication of Curcuma species (Danezis *et al.*, 2016).

3.2 Chemometrics

One of the factors contributing to the success of molecular spectroscopy and chromatographic fingerprinting as an analytical technique for authentication of Curcuma species is chemometrics software. Chemometrics is powerful tools widely used for the treatment of chemical data, especially for complex and overlapping molecular spectra (Bro *et al.*, 1997; Brereton, 2003). Chemometrics was firstly introduced in 1972 by Swante Wold, a scientist from Swedia, and Bruce R. Kowalski, scientist from the United States. Chemometrics is defined as the part of chemistry science using mathematics and statistics to: (1) design the procedure for optimal measurement of the assay and (2) to collect as much as chemical information by analyzing the data (Varmuza and Filzmoser, 2009). Chemometrics is an interdisciplinary method involving multivariate statistics, mathematical modeling, computer science, and analytical chemistry. One of the advantages of chemometrics is its ability in the analysis of multivariate data. Multivariate data are data resulted from the measurement of several variables in the same samples (Miller and Miller, 2010). International Chemometrics Society (ICS) has defined chemometrics 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 (Gemperline, 2006). For the authentication analysis of Curcuma species, chemometrics used for the treatment of spectra data is basically intended to two main purposes, namely qualitative purpose by making pattern recognition (classification and discrimination) and quantitative purpose by applying multivariate calibration model (Rohman and Che Man, 2012). Some spectra are also subjected to some processing before being used for making qualitative and quantitative analyses which include Savitzky-Golay-based derivatization, standard normal variate, mean centering, baseline corrections,

signal correction and compression, spectra normalizations, and multiplicative corrections. Pattern recognition can be either unsupervised such as discriminant analysis and canonical correlation analysis (CCA) or supervised methods such as principal component analysis (PCA) and cluster analysis. In addition, multivariate calibration for quantitative purpose includes stepwise multiple linear regression, multivariate curve resolution algorithms, genetic multivariate calibration, principle component regression and partial least square (Worley and Powers, 2016).

The chemometrics analysis is difficult to perform manually, fortunately, there are a number of user-friendly chemometrics software available to carry out calculations of complex data. Each program has its own features and advantages, for example, Unscrambler®, SIMCA®, SIRIUS®, and Pirouette® offer standard methods of multivariate analysis such as PCA, cluster analysis, PCR, PLS and Soft Independent Modelling of Class Analogy (SIMCA), but there is a little capacity to write personal programs. Conversely, Minitab® and Matlab® are designed to facilitate the writing of personal routines, and Grams®32 is particularly useful for routine quantitative analysis rather than for exploration of a data matrix by different pattern recognition technique (Miller and Miller, 2010; Gemperline, 2006). Currently, some instruments such as FTIR spectrophotometer are equipped with chemometrics software included as one package.

3.3 Authentication of Curcuma using molecular spectroscopies

The application of molecular spectra (UV-Vis, FTIR, FT-NIR, FT-Raman, and NMR) spectroscopies for discrimination and authentication of Curcuma species has been reported in several publications. Ultraviolet-visible (uv-vis) spectroscopy in combination with chemometrics of PCA and DA has been proposed as an authentication technique of Curcuma species, namely *C. longa*, *C. xanthorrhiza*, *C. aeruginosa* or black turmeric and *C. mangga* or mango ginger

(Rafi et al., 2018). The powders were subjected to extraction using sonication method with methanol as an extracting solvent for 40 mins. UV-Vis spectra of these four species were obtained in wavelength of 200-800 nm. The spectra were subjected to standard normal variate for spectral preprocessing. PCA and DA were performed using variables of absorbance values at the wavelength of 210-500 nm. The combination of UV-Vis and PCA, as well as UV-Vis and DA, could discriminate four species. DA offered clearer classification according to its species due to its capability to classify samples with an accuracy of 95.5% based on leave-one-out-cross-

validation compared to PCA.

Gad and Bouzabata (2017) have used molecular spectroscopies, namely uv-vis, FTIR and NMR in combination with chemometrics of PCA and HCA for complete profiling of metabolites in *C. longa* for assessing the quality control and separating the high-grade samples of *C. longa* with lower ones by determining the contents of curcuminoids. The results of curcuminoid contents were compared with those using HPLC. The results showed that score plot of PCA using variables of absorbances at the wavelength of 200-800 nm and variables of curcuminoid contents (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) revealed the same discriminatory pattern, in which 30 samples of *C. longa* from different commercial markets in Algeria and Egypt. The samples based on PC1 and PC2 could be separated into four main groups according to its total curcuminoids levels. However, FT-IR spectra at wavenumbers of 4000-400 cm^{-1} could not discriminate between the same species. In addition, using ^1H NMR, the variability of samples was more evident based on the regions (0.4–3 ppm) related to the composition of essential oils and fatty acid.

FTIR spectroscopy in mid-IR region (4000–400 cm^{-1}) combined with chemometrics of unsupervised pattern recognition of PCA and supervised pattern recognition of CVA has been developed for discrimination of *C. longa*, java turmeric (*C. xanthorrhiza*) and ginger (*Zingiber cassumunar*) having similar rhizome color and similar uses. Several spectral treatments including derivatization (first and second derivatives) and standard normal variate were used for optimization intended for the classifying three species. PCA using variables of FTIR spectral absorbances at wavenumbers of 2000–400 cm^{-1} was successfully used for classification of three species exploiting 10 PCs capable of describing 99.61% of total variance. CVA using similar variables also successfully discriminate samples with total variance from of two canonical variates (CVs) of 100%, with CV1 and CV2 of 86.7% 13.3%, respectively. This indicated that FTIR spectra and CVA could classify with the accuracy of 100% according to its groups. The results also showed that CVA offered clearer discrimination among samples evaluated than PCA (Rohaeti et al., 2015). The differentiation of *C. longa* and *C. xanthorrhiza* was also performed by FTIR spectroscopy combined with PLS by determination of curcumin contents in both Curcuma. The wavenumbers of 2000-950 cm^{-1} were selected for quantification of curcumin in both extracts and. The correlation between actual values of curcumin determined by HPLC and FTIR predicted values using FTIR spectroscopy combined with PLS in ethanolic extract of *C. longa* and *C. xanthorrhiza* at 2000-950 cm^{-1}

¹ revealed R² values of 0.96 and 0.99, respectively. The RMSEC values obtained are 0.299% and 0.089% for ethanolic extracts of *C. longa* and *C. xanthorrhiza*, respectively. Using FTIR spectroscopy-PLS, it can be stated that the contents of ethanolic extracts of *C. longa* were higher than those in *C. xanthorrhiza* indicating that this method could be used for authentication of Curcuma species (Rohman *et al.*, 2015). The contents of curcuminoid (curcumin and demethoxycurcumin) as determined by FTIR spectroscopy and multivariate calibration has been also used for identification of *C. xanthorrhiza* (Lestari *et al.*, 2017).

FT-Raman and FT-IR spectroscopic-based methods using ordinary least square were used for authentication of *C. longa* from methanyl yellow. Sample mixtures were prepared by mixing methanyl yellow at levels of 0.01, 1, 5, 10, 15, 20, 25 and 30% (w/w). FT-Raman and FT-IR spectra of these samples were scanned at 4000-400 cm⁻¹. For quantitative analysis of MY in turmeric, there is a good correlation between actual and FT-Raman predicted value using absorbance value at 1406 cm⁻¹ with the detection limit of 1% of MY. FTIR spectral regions were also evaluated for determination of MY, and finally, the absorbance ratio at 1140/966 cm⁻¹ was selected. The R² values obtained for such relationship were of 0.93 and 0.95, respectively using FT-Raman and FTIR spectroscopies, respectively (Dhakal *et al.*, 2016).

FT-Raman spectroscopy offered more sensitive than FT-IR in which FTIR spectroscopy has a detectable limit of 5%. These results indicated that both techniques could be used for authentication of turmeric powder from adulteration practice using methanyl yellow.

FTIR-ATR spectroscopy using wavenumber of 4000-600 cm⁻¹ combined with chemometrics of partial least square-discriminant analysis (PLS-DA) has been successfully used for rapid discrimination of *Curcuma longa* powder (turmeric) adulterated with *Curcuma zedoaria* and *Curcuma xanthorrhiza* in binary mixtures. The good of fit and good of predictivity of PLS-DA model were shown by the value of Q² (0.9558), R²X (0.9813), and R²Y (0.9746). Quantification of adulterants was successfully carried out using chemometrics of PLS. The PLS model showed good calibration models which are shown by higher R² and lower RMSEC value. External validation showed a high value of R² prediction and lower RMSEP value which confirms the validity of the PLS model (Rawar *et al.*, 2019).

Proton ¹H-NMR spectroscopy is an ideal method for metabolite fingerprinting, therefore this technique is a powerful technique to be used for authentication studies. ¹H-NMR spectra can provide 100-1000 variables available for multivariate data analysis (Verpoorte *et al.*,

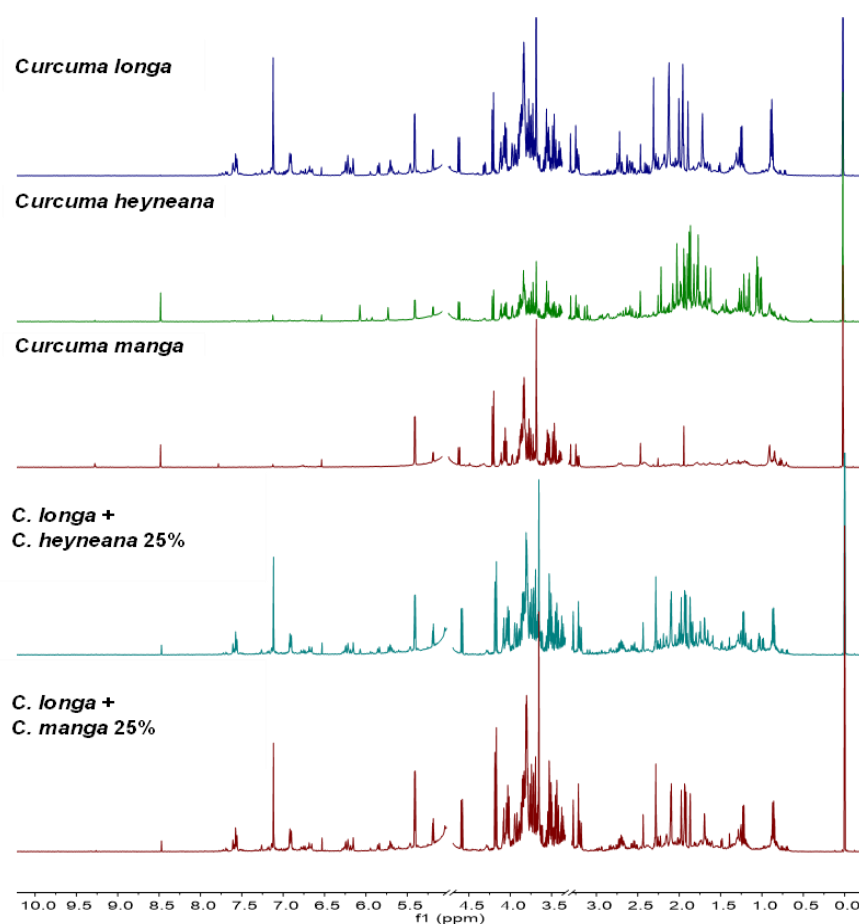


Figure 1. ¹H-NMR spectra of *Curcuma longa*, *Curcuma heyneana*, *Curcuma manga*, and adulterated *Curcuma longa* with 25% of adulterants concentration (Windarsih *et al.*, 2018b).

2007). Windarsih *et al.* (2018a) have used $^1\text{H-NMR}$ spectra combined with PCA and orthogonal projections to latent structures-discriminant analysis (OPLS-DA) for authentication of *C. longa* from *C. manga*. $^1\text{H-NMR}$ spectra of *C. longa*, *C. heyneana* and *C. manga* exhibited characteristic profiles to differentiate between *C. longa* from *C. heyneana* and *C. manga* using certain regions of chemical shift (Figure 1). The difference in the chemical shift was caused by its metabolite contents present in Curcuma species. In the regions of 0.00-3.00 ppm, *C. longa* has more signals with higher intensities, while *C. manga* revealed few signals with low intensities. *C. longa* also has more signals and higher intensities than *C. manga* in regions of 5.50-10.00 ppm, and no signals observed after region of 8.00 ppm. The regions of 0.30-3.00 ppm, 3.00-6.00 ppm, and 6.00-10.00 ppm corresponded to essential oils/ fatty acid, carbohydrates, and aromatic compounds present in evaluated samples.

OPLS-DA offered better separation than PCA for discrimination of *C. longa* and *C. longa* adulterated with *C. manga* and with *C. heyneana* as shown in Figure 2. $^1\text{H-NMR}$ spectra at regions of 6.00-8.00 combined with OPLS-DA has been successfully used for classification of *C. longa* and *C. longa* adulterated with *C. manga*. $^1\text{H-NMR}$ combined with OPLS-DA has also used for differentiation of authentic and adulterated *C. longa* with *C. heyneana*. The variables used are peaks with certain chemical shifts at optimized $^1\text{H-NMR}$ spectra of authentic and adulterated *C. longa*. All of the authentic *C. longa* samples were clearly separated from the adulterated ones. The multivariate calibration of partial least square (PLS) was successfully applied to predict of adulterants in *C. longa*. The lower RMSEC and RMSEP values of 0.94% and 0.83%, respectively for adulterated *C. longa* with *C. heyneana* indicated the good of accuracy and precision of the calibration models to authentication studies (Windarsih *et al.*, 2018b).

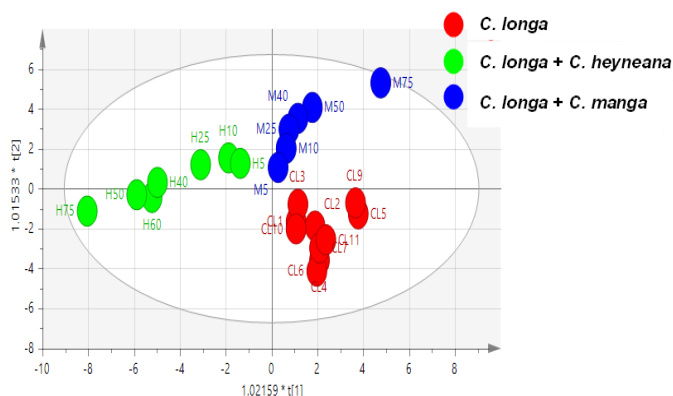


Figure 2. OPLS-DA score plot of *Curcuma longa* and adulterated *Curcuma longa* with *Curcuma heyneana* and *Curcuma manga* (Windarsih *et al.*, 2018b)

The application of metabolite fingerprinting based on $^1\text{H-NMR}$ for the authentication of *C. xanthorrhiza*

adulterated with *Zingiber cassumunar* has been reported by Wijayanti *et al.* (2019). The chemometrics used as PCA and PLS-DA. A diverse group of metabolites could be detected by $^1\text{H-NMR}$ spectroscopy. PCA using the chemical shift in $^1\text{H-NMR}$ spectra of the plant extracts as variables clearly discriminated pure *C. xanthorrhiza* extracts from different origins and *C. xanthorrhiza* extract adulterated with *Z. cassumunar*. PLS-DA employed to enhance the separation obtained from the PCA model resulted in well separation and good classification of pure *C. xanthorrhiza* from the adulterated ones. The developed method could be a useful and powerful tool to assess adulteration practice and to evaluate the authentication of *C. xanthorrhiza* extracts.

3.4 Authentication of *Curcuma* species using chromatography

Due to its capability to separate components of interest present in Curcuma species, chromatographic-based techniques such as thin layer, liquid, and gas chromatography in combination with chemometrics evolved as emerging techniques for authentication studies (Esteki *et al.*, 2018). Chromatographic methods equipped with numerous detectors have been used for identification of adulteration practices by quantification of specific markers (compounds) present in genuine and adulterated samples (Esteki *et al.*, 2017). Most chromatographic methods intended to Curcuma species authentication are developed for analysis of curcuminoid in *C. longa* and *C. xanthorrhiza*.

Rafi *et al.* (2011) have developed TLC fingerprints for differentiation of three herbs, namely Turmeric (*Curcuma longa*), java turmeric (*Curcuma xanthorrhiza*) and cassumunar ginger (*Zingiber cassumunar*). The visualization of separated zones was performed using visible and UV lights at 254 and 366 nm, respectively. The pattern of TLC fingerprint was different among three herbs evaluated exhibiting specific marker zones respectively. For example, turmeric was characterized by 7 zones at Rf of 0.05–0.97 under UV 254. This result indicated that TLC fingerprint can be used for discrimination and authentication of three herbs tested.

HPLC is a common method used for quantitative analysis of curcuminoid either for authentication or quality control purposes (Siregar *et al.*, 2017). HPLC using fingerprint profiles combined with supervised pattern recognition of DA has been exploited for authentication and discrimination of *C. xanthorrhiza* from *C. longa* by determining the contents of curcuminoids. The results showed that curcuminoid contents in *C. longa* were higher than those in *C. xanthorrhiza*, as a consequence both species could be

discriminated and authenticated based on curcuminoid levels. In addition, the discrimination and authentication of both species were also achieved based on HPLC fingerprint chromatograms using curcuminoid components as marker peaks. Combined with DA, HPLC fingerprint offered excellent result allowing two species to be separated and correctly classified (Rafi *et al.*, 2015).

4. Conclusion

The authenticity of *Curcuma* species, especially *Curcuma longa* (turmeric) and *Curcuma xanthorrhiza* (javanese turmeric) used as important components in some traditional medicine is very important. Some analytical methods based and molecular spectra are proposed, developed, validated and used for authentication of *C.longa* and *C. xanthorrhiza*. Combined with chemometrics techniques, molecular spectra offered rapid, reliable and no excessive sample preparation for authentication of *Curcuma*.

Conflict of Interest

The author declares that no conflict of interest

Acknowledgements

The publication of this paper was supported by scheme World Class Research (WCR) 2020 from the Ministry of Research and Technology with contract number: 3848/UN1/DITLIT/DIT-LIT/PT/2020

References

- Bar-Sela, G., Epelbaum, R. and Schaffer, M. (2010). Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. *Current Medicinal Chemistry*, 17(3), 190–197. <https://doi.org/10.2174/092986710790149738>
- Brereton, R.G. (2003). *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*. UK: John Wiley and Sons. <https://doi.org/10.1002/0470863242>
- Bro, R., Jerome, J.W.Jr., Paul, R.M. and Kowalski, B.R. (1997). Review of chemometrics applied to spectroscopy: 1985–95, Part 3—Multi-way analysis. *Applied Spectroscopy Reviews*, 32(3), 237–261. <https://doi.org/10.1080/05704929708003315>
- Bumrah, G.S. and Sharma, R.M. (2016). Raman spectroscopy – Basic principle, instrumentation and selected applications for the characterization of drugs of abuse. *Egyptian Journal of Forensic Sciences*, 6(3), 209–215. <https://doi.org/10.1016/j.ejfs.2015.06.001>
- Chen, D.D., Xie, X.F., Ao, H., Liu, J.L. and Peng, C. (2017). Raman spectroscopy in quality control of Chinese herbal medicine. *Journal of The Chinese Medical Association*, 80(5), 288-296. <https://doi.org/10.1016/j.jcma.2016.11.009>
- Cousins, M., Adelberg, J, Chen., F. and Rieck, J. (2007). Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric *Curcuma longa* L. grown *in vitro*. *Industrial Crops and Products*, 25 (2), 129–35. <https://doi.org/10.1016/j.indcrop.2006.08.004>
- Cozzolino, D. (2015). The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. *Molecules*, 20(1), 726-37. <https://doi.org/10.3390/molecules20010726>
- Dais, P. and Hatzakis, E. (2013). Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. *Analytica Chimica Acta*, 765, 1-27. <https://doi.org/10.1016/j.aca.2012.12.003>
- Danezis, G.P., Tsagkaris, A.S., Camin, F., Brusica, V. and Georgiou, C.A. (2016). Food authentication: Techniques, trends & emerging approaches. *Trends in Analytical Chemistry*, 85(Part A), 123-32. <https://doi.org/10.1016/j.trac.2016.02.026>
- Dhakal, S., Chao, K., Schmidt, W., Qin, J., Kim, M. and Chan, D. (2016). Evaluation of Turmeric Powder Adulterated with Metanil Yellow Using FT-Raman and FT-IR Spectroscopy. *Foods*, 5(2), 36-42. <https://doi.org/10.3390/foods5020036>
- Esteki, M., Farajmand, B., Kolahderazi, Y. and Simal-Gandara, J. (2017). Chromatographic fingerprinting with multivariate data analysis for detection and quantification of apricot kernel in almond powder. *Food Analytical Methods*, 10(10), 3312-3320. <https://doi.org/10.1007/s12161-017-0903-5>
- Esteki, M., Simal-Gandara, J., Shahsavari, Z., Zandbaaf, S., Dashtaki, E. and Heyden, Y.V. (2018). A review on the application of chromatographic methods, coupled to chemometrics for food authentication. *Food Control*, 93, 165-182. <https://doi.org/10.1016/j.foodcont.2018.06.015>
- Farzaei, M.H., Zobeiri, M., Parvizi, F., El-Senduny, F.F., Marmouzi, I., Coy-Barrera, E., Naseri, R., Nabavi, S.M., Rahimi, R. and Abdollahi, M. (2018). Curcumin in liver diseases: A systematic review of the cellular mechanism of oxidative stress and clinical perspective. *Nutrients*, 10(7), 855. <https://doi.org/10.3390/nu10070855>
- Gad, H.A. and Bouzabata, A. (2017). Application of chemometrics in quality control of Turmeric (*Curcuma longa*) based on Ultra-violet, Fourier

- transform-infrared and ^1H NMR spectroscopy. *Food Chemistry*, 237, 857–864. <https://doi.org/10.1016/j.foodchem.2017.06.022>
- Gemperline, P. (2006). *Practical Guide to Chemometrics*. Boca Raton, Florida, USA: CRC Press. <https://doi.org/10.1201/9781420018301>
- Gendrin, C., Roggo, Y. and Collet, C. (2008). Pharmaceutical applications of vibrational chemical imaging and chemometrics: A review. *Journal of Pharmaceutical and Biomedical Analysis*, 48(3), 533–553. <https://doi.org/10.1016/j.jpba.2008.08.014>
- Griffiths, P.R. and Chalmers, J.M. (Eds.). (1999). *Handbook of vibrational spectroscopy*. New York: John Wiley and Sons.
- Hannah, R.W. (2002). Standard sampling techniques for infrared spectroscopy. In Chalmers, J.M. and Griffiths, P.R. (Eds.). *Handbook of vibrational spectroscopy*. Vol. 2, p. 933-952. UK: John Wiley and Sons.
- Honda, S., Aoki, F., Tanaka, H., Kishida, H., Nishiyama, T., Okada, S., Matsumoto, I., Abe K. and Mae, T. (2006). Effects of ingested turmeric oleoresin on glucose and lipid metabolisms in obese diabetic mice: a DNA microarray study. *Journal of Agricultural and Food Chemistry*, 54, 9055–9062. <https://doi.org/10.1021/jf061788t>
- Jantan, I., Saputri, F.C., Qaisar, M.N. and Buang, F. (2012). Correlation between chemical composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human low-density lipoprotein oxidation. *Evidence Based Complementary Alternative Medicine*, 2012, 1–10. <https://doi.org/10.1155/2012/438356>
- Jurenka, J.S. (2009). Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Alternative Medicine Review a Journal of Clinical Therapeutic*, 14, 141–153.
- Kalantri, P.P., Somani, R.R. and Makhija, T. (2010). Raman spectroscopy: a potential technique in analysis of pharmaceuticals. *Der Chemica Sinica*, 1, 1-12.
- Lestari, H.P., Martono, S., Wulandari, R. and Rohman, A. (2017). Simultaneous analysis of Curcumin and demethoxycurcumin in *Curcuma xanthorrhiza* using FTIR spectroscopy and chemometrics. *International Food Research Journal*, 24, 2097-2101.
- Li, S., Yuan, W., Deng, G., Wang, P., Yang, P. and Aggarwal, B. (2011). Chemical composition and product quality control of turmeric *Curcuma longa* L. *Pharmaceutical Crops*, 2, 28–54. <https://doi.org/10.2174/2210290601102010028>
- Miller, J.N. and Miller, J.C. (2010). *Statistics and Chemometrics for Analytical Chemistry*, 6th ed. Harlow, USA: Prentice Hall,
- Mohanty, I., Singh Arya, D., Dinda, A., Joshi, S., Talwar, K.K. and Gupta, S.K. (2004). Protective effects of *Curcuma longa* on ischemia-reperfusion induced myocardial injuries and their mechanisms. *Life Sciences*, 75(14), 1701–1711. <https://doi.org/10.1016/j.lfs.2004.02.032>
- Pavia, D.L., Lampman, G.M. and Kriz-jr G.S. (2009). *Introduction to Spectroscopy: A Guide for Students of Organic Chemistry*. Philadelphia, USA: W.B. Saunders Company.
- Rafi, M., Rohaeti, E., Miftahudin, A. and Darusman, L.K. (2011). Differentiation of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar* by thin layer chromatography fingerprint analysis. *Indonesian Journal of Chemistry*, 11, 71–74. <https://doi.org/10.22146/ijc.21423>
- Rafi, M., Wulansari, L., Heryanto, R., Darusman, L.K., Lim, L.W. and Takeuchi, T. (2015). Curcuminoid's content and fingerprint analysis for authentication and discrimination of *Curcuma xanthorrhiza* from *Curcuma longa* by high-performance liquid chromatography-diode array detector. *Food Analytical Methods*, 8(9), 2185-2193. <https://doi.org/10.1007/s12161-015-0110-1>
- Rafi, M., Jannah, R., Heryanto, R., Kautsar, A. and Septaningsih, D.A. (2018). UV-Vis spectroscopy and chemometrics as a tool for identification and discrimination of four *Curcuma* species. *International Food Research Journal*, 25, 643-8.
- Ramdani, E.D., Marlupi, U.D., Sinambela, J. and Tjandrawinata, R.R. (2016). A new method of xanthorrhizol isolation from the rhizome extract of *Curcuma xanthorrhiza*. *Scholars Academic Journal of Biosciences*, 4, 732–737.
- Rawar, E.A., Susidarti, R.A. and Rohman, A. (2019). Rapid authentication of turmeric powder adulterated with *Curcuma zedoaria* and *Curcuma xanthorrhiza* using FTIR-ATR spectroscopy and chemometrics. *International Journal of Applied Pharmaceutics*, 11 (5), 216-221. <https://doi.org/10.22159/ijap.2019v11i5.33701>
- Remya, R., Syamkumar, S. and Sasikumar, B. (2004). Isolation and amplification of DNA from turmeric powder. *British Food Journal*, 106, 673–678. <https://doi.org/10.1108/00070700410558201>
- Rohaeti, E., Rafi, M., Syafitri, U.D. and Heryanto, R. (2015). Fourier transform infrared spectroscopy combined with chemometrics for discrimination of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber*

- cassumunar*. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 137, 1244–1249. <https://doi.org/10.1016/j.saa.2014.08.139>
- Rohman, A. and Che Man, Y.B. (2012). Application of Fourier transform infrared spectroscopy for authentication of functional food oils. *Applied Spectroscopy Reviews*, 47(1), 1-13. <https://doi.org/10.1080/05704928.2011.619020>
- Rohman, A., Nugroho, A., Lukitaningsih, E. and Sudjadi. (2014). Application of vibrational spectroscopy in combination with chemometrics techniques for authentication of herbal medicine. *Applied Spectroscopy Reviews*, 49(8), 603-613. <https://doi.org/10.1080/05704928.2014.882347>
- Rohman, A., Sudjadi, S., Devi, D. and Nugroho, A. (2015). Analysis of Curcumin in *Curcuma longa* and *Curcuma xanthorrhiza* using FTIR spectroscopy and chemometrics. *Research Journal of Medicinal Plant*, 9, 179-186. <https://doi.org/10.3923/rjmp.2015.179.186>
- Rouessac, F. and Rouessac, A. (2007). *Chemical Analysis: Modern Instrumentation Methods and Techniques*. 2nd ed. Chichester, England: John Wiley and Sons Ltd.
- Settle, F.A. (1997). *Handbook of Instrumental Techniques for Analytical Chemistry*. New Jersey, USA: Prentice, Inc,
- Siregar, C., Prabaningdyah, N.K., Choiri, S., Riyanto, S. and Rohman, A. (2017). Optimization of HPLC using central composite design for determination of Curcumin and Demethoxycurcumin in tablet dosage form. *Dhaka University Journal of Pharmaceutical Sciences*, 16(2), 137-145. <https://doi.org/10.3329/dujps.v16i2.35248>
- Skoog, D.A., Holler, F.J. and Crouch, S.R. (2007). *Principles of Instrumental Analysis*, 6th ed. Belmont, USA: Thomson Higher Education.
- Tajuddin, T., Cartealy, I.C., Safarrida, A., Purwoko, D., Zulaeha, S. and Ardiyani M. (2011). Identification on Indonesian Accessions of *Curcuma xanthorrhiza* Roxb. using AFLP Markers, presented at Proceeding the International Conference on Bioscience and Biotechnology 2011. Kalijaga, Yogyakarta: Biology Department, Faculty of Science and Technology, State Islamic University Sunan
- Varmuza, K. and Filzmoser, P. (2009). *Introduction to multivariate statistical analysis in chemometrics*. Boca Raton, USA: CRC Press.
- Verpoorte, R., Choi, Y.H. and Kim, H.K. (2007). NMR-based metabolomics at work in phytochemistry. *Phytochemistry Reviews*, 6, 3-14. <https://doi.org/10.1007/s11101-006-9031-3>
- Wang, P., Sun, J., Zhang, T. and Liu, W. (2016). Vibrational spectroscopic approaches for the quality evaluation and authentication of virgin olive oil. *Applied Spectroscopy Reviews*, 51(10), 763-790. <https://doi.org/10.1080/05704928.2016.1176034>
- Wartewig, S. and Neubert, R.H.H. (2005). Pharmaceutical applications of Mid-IR and Raman spectroscopy. *Advanced Drug Delivery Reviews*, 57(8), 1144–1170. <https://doi.org/10.1016/j.addr.2005.01.022>
- Wijayanti, T., Riyanto, S., Lukitaningsih, E. and Rohman, A. (2019). Application of ¹H-NMR spectra and multivariate analysis for the authentication of *Curcuma xanthorrhiza* from *Zingiber cassumunar*. *International Journal of Applied Pharmaceutics*, 11(4), 341-344. <https://doi.org/10.22159/ijap.2019v11i4.32417>
- Windarsih, A., Rohman, A. and Swasono, R.T. (2018a). Application of H-NMR metabolite fingerprinting and chemometrics for the authentication of *Curcuma longa* adulterated with *Curcuma manga*. *Journal of Applied Pharmaceutical Science*, 8(6), 75-81. <https://doi.org/10.7324/JAPS.2018.8610>
- Windarsih, A., Rohman, A. and Swasono, R.T. (2018b). Authentication of turmeric using proton-nuclear magnetic resonance spectroscopy and multivariate analysis. *International Journal of Applied Pharmaceutics*, 10(6), 174-180. <https://doi.org/10.22159/ijap.2018v10i6.29014>
- Worley, B. and Powers, R. (2016). PCA as a practical indicator of OPLS-DA model reliability. *Current Metabolomics*, 4(2), 97-103. <https://doi.org/10.2174/2213235X04666160613122429>