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Original Article

RAPID AUTHENTICATION OF TURMERIC POWDER ADULTERATED WITH *CURCUMA ZEDOARIA* AND *CURCUMA XANTHORRHIZA* USING FTIR-ATR SPECTROSCOPY AND CHEMOMETRICS

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ABSTRACT

Objective: The objective of this study is to develop a rapid, simple, non-destructive and inexpensive analytical method using Fourier Transform Infrared (FTIR) spectroscopy with Attenuated Total Reflection (ATR) as a sampling technique, combined with chemometrics for authentication of turmeric powder adulterated with *Curcuma zedoaria* and *Curcuma xanthorrhiza*.

Methods: Turmeric powder is placed above the diamond crystal in ATR compartment. Spectra are scanned in the absorbance mode from 4000 to 600 cm^{-1}. The obtained spectra is further analyzed by Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA), and Partial Least Square Regression (PLS-R).

Results: PCA score plot shows that *Curcuma longa, Curcuma zedoaria,* and *Curcuma xanthorrhiza* can be discriminated well. PLS-DA can be used to build the model for classification between pure turmeric powder and adulterated powder with the value of Q^2, R^2X, and R^2Y of 0.9558, 0.9813, and 0.9746, respectively. The good calibration model for quantification of each adulterant is obtained by PLS-R with R^2 value more than 0.99 and lower RMSEC value. The models have been validated by internal and external validations, which resulting in the high R^2 value and low RMSEP value which indicates that both models are accurate and precise.

Conclusion: The combination of FTIR-ATR spectroscopy and chemometrics can be used to authenticate turmeric powder adulterated with *Curcuma zedoaria* and *Curcuma xanthorrhiza.*

Keywords: *Curcuma longa*, *Curcuma zedoaria*, *Curcuma xanthorrhiza*, FTIR spectroscopy, Chemometrics

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INTRODUCTION

Turmeric (*Curcuma longa)*, the most widely used species of *Curcuma*, is an important commodity in the international trade of spices and medicinal plants. It is a native herb from Southeast Asia and is extensively cultivated in tropical and subtropical regions [1]. In Asia, it has long been used traditionally as a medicinal herb that possesses a wide range of pharmacological activities, such as antioxidant [2], anti-inflammatory [3], anti-protozoal [4], and anti-influenza [5]. The main compound of turmeric, which is responsible for its pharmacological activities and its yellow color, is curcuminoids consisting of curcumin, demethoxycurcumin, and bisdemethoxycurcumin [6]. The curcuminoid content of *C. longa* is relatively higher than that of other *Curcuma* species, such as *C. xanthorrhiza* [7]. For this reason, turmeric has been extensively used as a food coloring and as a raw ingredient of herbal supplements [1].

Due to the high demand for turmeric in international trade, adulteration practices often happen in order to increase profit. Turmeric in powder form is probably adulterated by starches, synthetic dyes, curcumin synthetic, and closely related species [1]. Physically, the rhizome of *Curcuma longa* can be differentiated with another rhizome of *Curcuma* species, such as *Curcuma zedoaria*, *Curcuma xanthorrhiza*, *Curcuma heynena*, *Curcuma manga*, etc. However, due to the similar color of the rhizome, it is difficult to differentiate among them in the powder form [8]. Consequently, the adulteration of turmeric powder by closely related species can happen intentionally and unintentionally. Therefore, the presence of other *Curcuma* species, such as *C. zedoaria* and *C. xanthorhhiza*, has been detected in turmeric powder sold in India [9].

Some analytical methods have been developed for authenticating medicinal plants, including fingerprint analysis using High-Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) combined with chemometrics [7], metabolomics technique using Proton Nuclear Magnetic Resonance (H-NMR) combined with multivariate analysis [10], and DNA based approach using DNA Barcoding [9], Random Amplified Polymorphic DNA (RAPD) [11], and Sequence Characterized Amplified Region (SCAR) [12]. The main disadvantage of these methods is that several steps are needed for sample preparation, making the process slow and costly. Meanwhile, FTIR spectroscopy has been used for authentication of some medicinal plants due to it's rapid, simple, non-destructive, and cost-effective technique [13].

IR spectra have multivariate data so that chemometrics is needed to analyze them. Some multivariate analyses often used to interpret IR spectra are Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA), and Partial Least Square Regression (PLS-R) [14]. PCA is the most popular unsupervised pattern recognition, which can detect similarities without prior grouping [15]. PLS-DA is a classification method based on the PLS regression algorithm in order to make a class assignment [16]. PLS regression uses a linear combination of the predictor variables obtained by giving extra weight variables which have a high correlation with response variables so that they will be more effective for quantification [17].

The objective of this study was to develop a rapid, simple, non-destructive, and cost-effective analytical method to authenticate turmeric powder from *Curcuma zedoaria* and *Curcuma xanthorrhiza* by using FTIR spectroscopy combined with chemometrics, namely PCA, PLS-DA, and PLS-R.

MATERIALS AND METHODS

The rhizomes of *Curcuma longa* were obtained from Sleman, Bandung, Wonogiri, Wates, Kulonprogo, and Gunungkidul, Java Island, Indonesia. Plant determination of these rhizomes was performed in Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta. The rhizomes of *Curcuma xanthorrhiza* and *Curcuma zedoaria* were obtained from

Sukolilo and Kulonprogo, respectively. The rhizomes were peeled, cleaned and sliced into small pieces. They were dried and then pulverized into powder.

FTIR spectroscopy measurement

The FTIR spectra of samples were obtained using FTIR-ATR Nicolet iS10 equipped with Deuterated Tri-Glycine Sulfate (DTGS) as the element detector and potassium bromide (KBr) as the beam splitter. The instrument was integrated with OMNIC software for FTIR spectra processing. The measurements were directly carried out by introducing sample powder above the diamond crystal on ATR sample compartment and was recorded in the region 4000-650 cm^{-1} with 32 scans/min and resolution of 8 cm^{-1} at controlled room temperature (25 °C). The powders of each *Curcuma* species and adulterated turmeric powders were scanned to be further analyzed by PCA. The pure turmeric powder and the adulterated turmeric powder by *Curcuma xanthorrhiza* and *Curcuma zedoaria* were prepared accurately in the concentration ranges of 10.0-100.0 % (wt/wt). Samples were divided into 3 samples set, i.e., training set (for calibration), validation set, and test set for being further analyzed by PLS-DA and PLS-R.

Chemometric analysis

The adds in XLSTAT 2018 Addison integrated with Microsoft Excel 2013 software is used to perform PCA and PLS-DA. TQ Analyst software was used to analyse IR spectra by PLS regression. Frequency regions selected were in the fingerprint area (1630-1200 cm^{-1}).

RESULTS AND DISCUSSION

FT-MIR spectral analysis

Medicinal plants consist of various chemical compounds. Some functional groups of them can absorb energy from IR light in a certain wavelength number [13]. Turmeric powder contains curcuminoids (curcumin, demethoxycurcumin, bisdemethoxy-curcumin) [6]. The structure of each curcuminoid is shown in fig. 1.

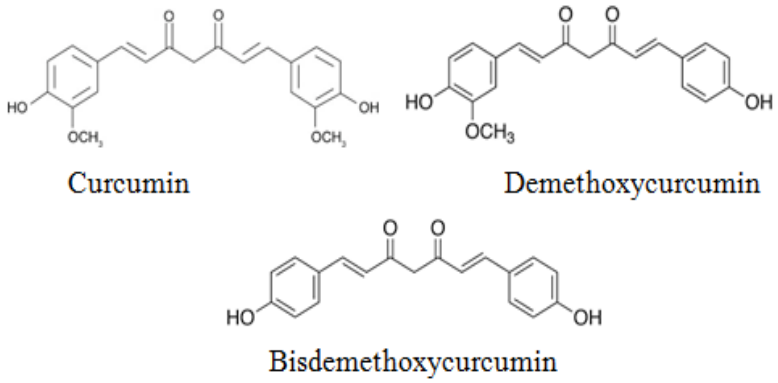

Fig. 1: The chemical structures of Curcuminoids (Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin)

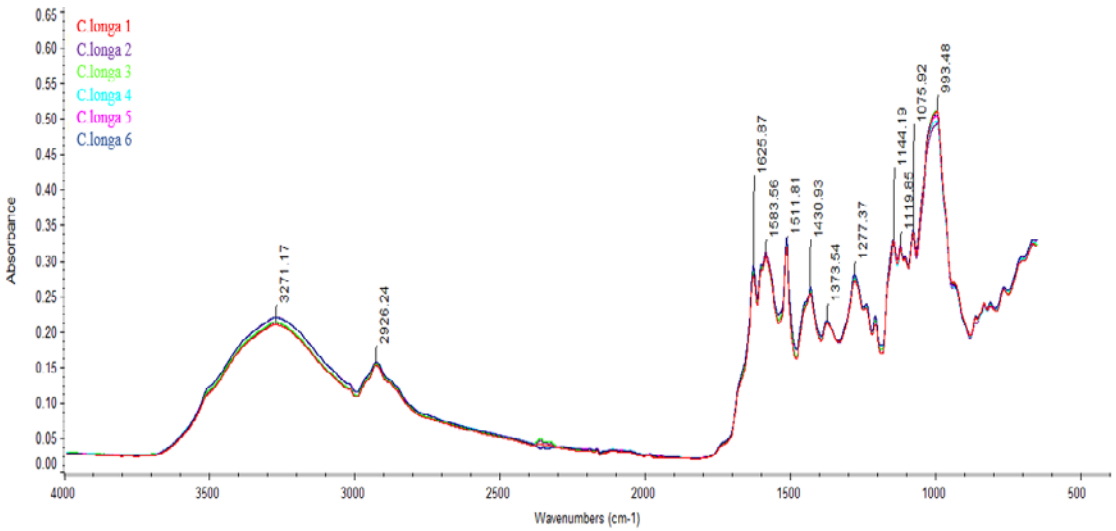

Fig. 2: FTIR spectra of *Curcuma longa* from six regions. 1. Sleman, 2. Bandung, 3. Wonogiri, 4. Wates, 5. Kulonprogo, 6. Gunungkidul

Table 1: The characteristics peak in *Curcuma* species's FTIR spectra [18]

No.	Wavelength number (cm^{-1})	Functional group
1	~3400	O-H absorption
2	2800-3000	Methyl (-CH_3) and methylene (-CH_2) symmetric and asymmetric vibration
3	1740-1680	C=O absorption
4	~1510	The aromatic skeletal stretching vibration
5	~1030	C-OH stretching vibration

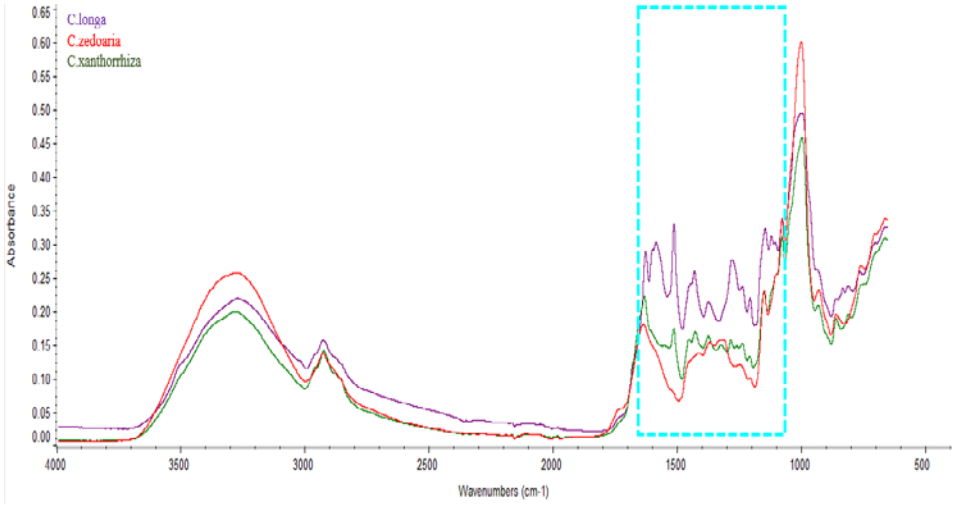

Fig. 3: The IR spectra of *Curcuma longa*, *Curcuma xanthorrhiza*, and *Curcuma zedoaria*

Fig. 2 shows that the IR spectra from six regions in Java Island have high similarities. Fig. 3 shows the IR spectra between *Curcuma longa*, *Curcuma xanthorrhiza*, and *Curcuma zedoaria* are significantly different in 1650-1200 cm^{-1}. The different curcuminoid content of those three species cause variations in their peak positions and intensities. Interpretation of functional groups from each peak is described in table 1.

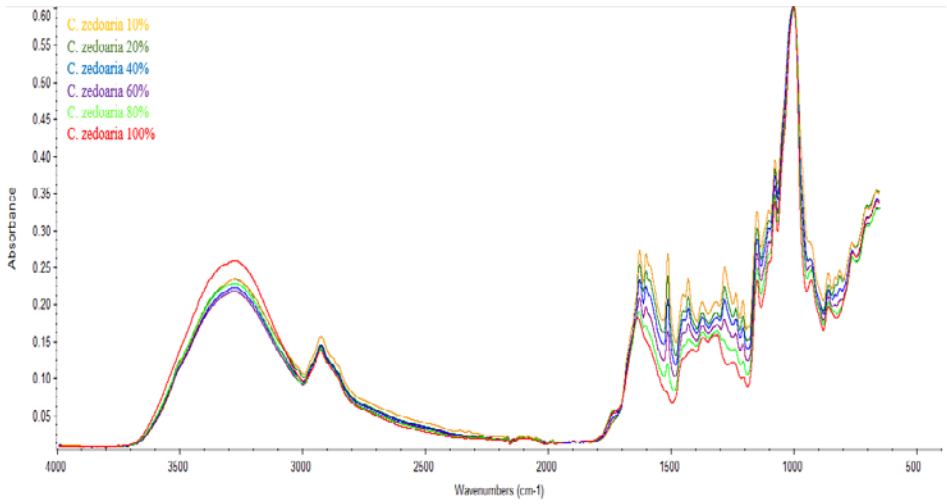

Fig. 4: FTIR spectra of pure and adulterated *Curcuma longa* with *Curcuma zedoaria* by various adulterant concentrations

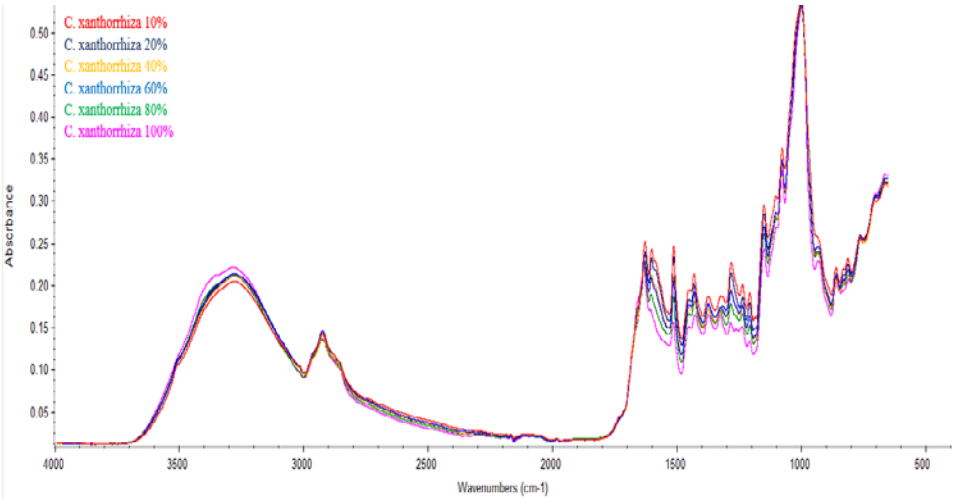

Fig. 5: FTIR spectra of pure and adulterated *Curcuma longa* with *Curcuma xanthorrhiza* by various adulterant concentrations

Fig. 4 and fig. 5 shows that the increasing proportion of adulterant influences the change at wavelength number 1650-1200 cm^{-1}. FTIR spectra contains thousands absorbance values from a lot of wavelength numbers as variable responses. Due to the complexity of the data, it is difficult to interpret the data visually. Multivariate analysis, such as PCA, PLS-DA, and PLS-R are needed to analyze these data.

Discrimination of *Curcuma longa*, *Curcuma zedoaria*, and *Curcuma xanthorrhiza* using PCA

Exploratory data analysis (EDA) is needed to be done before building a calibration model for quantification or classification. EDA is able to detect the outliers, recognize patterns, and evaluate the correlation between variables and classes [16]. PCA is the most used method for EDA and the popular unsupervised pattern recognition by reducing the data and extracting the information in order to find a combination of variables or factors for describing major trends in a dataset [15]. In this study, PCA was employed to discriminate the samples according to the species based on the FTIR spectra in the region of 1650-1200 cm^{-1}. This region was selected because it was complex and full of information with many vibrations attributed to the chemical components in all samples. Fig. 5 shows that *Curcuma longa*, *Curcuma xanthorrhiza*, *Curcuma zedoaria,* and the adulterated turmeric powder could be discriminated well.

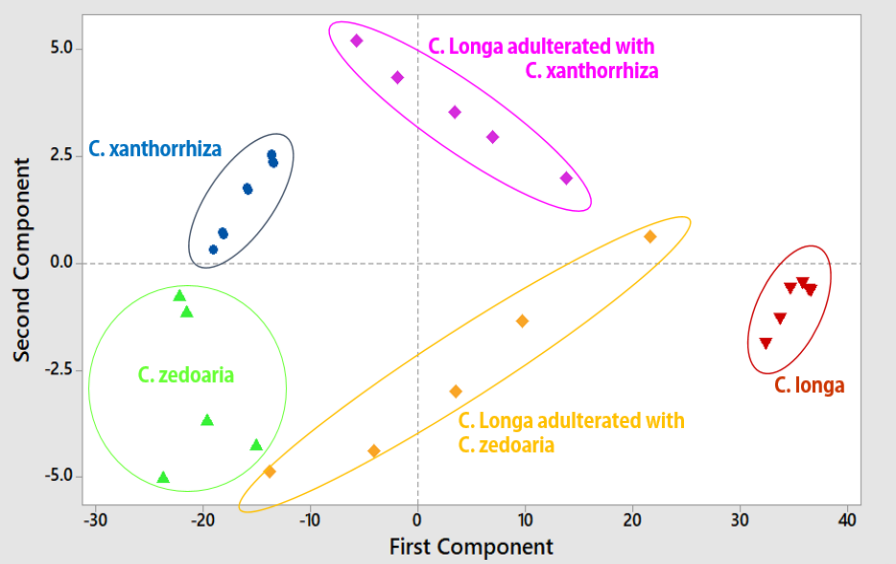

Fig. 5: PCA score plot of *Curcuma longa*, *Curcuma xanthorrhiza*, *Curcuma zedoaria,* and the adulterated turmeric powder

Authentication of *Curcuma longa* from *Curcuma zedoaria* and *Curcuma xanthorrhiza* using PLS-DA

PCA is an unsupervised pattern recognition which makes a classification based on the similarity of the principal component (PC) score [15]. Otherwise, PLS-DA is a supervised pattern recognition which classifies objects based on a calibration model built by latents variable possessing high correlation with variable responses described by LV scores and loadings [16]. PLS-DA was performed to discriminate between authentic pure turmeric powders and adulterated turmeric powders with *Curcuma zedoaria* and *Curcuma xanthorrhiza*. The quality of the classification model was determined by the cumulative value of Q^2, R^2X, and R^2Y [10]. R^2X and R^2Y values describe the correlation between X and Y. R^2X and R^2Y indicating the goodness of fit of the model was close to 1. The cumulative value of Q^2 determines the predictive capability of model. Q^2 value indicates the goodness of predictivity of the model is higher than 0.5. The factors can describe 94.00% variances which occurred in the spectral data with Q^2, R^2X, and R^2Y are 0.940, 0.967, and 0.981, respectively.

Table 2: Confusion matrix of PLS-DA performances for discrimination of *Curcuma longa* from *Curcuma zedoaria* and *Curcuma xanthorrhiza* in binary mixture with *Curcuma longa*

From/To	CL	Fake	Total	Correct (%)
CL	2	0	2	100
Fake	0	17	17	100
Total	2	17	19	100

PLS-DA was employed to discriminate between pure turmeric powder and adulterated turmeric powder with *Curcuma zedoaria* and *Curcuma xanthorrhiza*. The pure turmeric powder was labeled as CL, whereas, the adulterated one was labeled as "Fake". Table 2 shows the performance of PLS-DA in discriminating samples by confusion matrix. The value of percentage correctness of 100% indicates that all samples are classified correctly to the appropriate group. The threshold of acceptance is set by 0.50. Sample will be assigned to the group which the P-value more than 0.50. Table 3 showed that 10% adulterated turmeric powder by both *Curcuma zedoaria* and *Curcuma xanthorrhiza* labelled by "fake". The results in table 2 and table 3 showed the sensitivity and specificity of the model.

FTIR and PLS regression for quantification of adulterants in *Curcuma longa*

Partial least square (PLS) regression is one of the linear regression technique in the multivariate analysis by transforming the original variable to latent variables which have a high correlation with variable response [17]. Quantification of adulterants of *Curcuma zedoaria* and *Curcuma xanthorrhiza* in binary mixtures with *Curcuma longa* was performed using the PLS calibration model in concentration range of 10-100% wt/wt. The good calibration model was represented by the low root mean square error of calibration (RMSEC) value and high R^2 value [16]. RMSEC is a parameter to evaluate the occurrence of overfitting and to make a prior assumption that the relationship is in a linear way [10]. RMSEC value and R^2 value of *Curcuma zedoaria* in

Curcuma longa are 0.0106 and 0.9995, respectively. RMSEC value and R^2 value of *Curcuma xanthorrhiza* in *Curcuma longa* are 0.00794 and 0.9997, respectively. The calibration model was then validated by internal and external validations. Root mean square error of prediction (RMSEP) is a parameter to evaluate the goodness of predictive performance [16]. RMSEP value and R^2 value of *Curcuma zedoaria* in *Curcuma longa* are 0.0465 and 0.9965, respectively. RMSEP value and R^2value of *Curcuma xanthorrhiza* in *Curcuma longa* are 0.0790 and 0.9970, respectively.

PLS regression model for *Curcuma zedoaria* in *Curcuma longa* and *Curcuma xanthorrhiza* in *Curcuma longa* were calibrated and validated resulting in high accuracy and precision so that they could be used to quantitate *Curcuma zedoaria* and *Curcuma xanthorrhiza* in turmeric powder. Fig. 6 shows the correlation between the actual and the calculated responses of the *Curcuma zedoaria* in a binary mixture with *Curcuma longa* with the equation of y = 1.0616x–6.6849 and the R^2 value was 0.9944. Fig. 7 shows the correlation between the actual and the calculated responses of the *Curcuma xanthorrhiza* in a binary mixture with *Curcuma longa* with the equation of y = 1.0622x–8.0466 and the R^2 value was 0.9917. The result shows that a good relationship was obtained between the actual and calculated responses of both models.

Table 3: Discrimination validity of pure *Curcuma longa* in binary mixture with *Curcuma zedoaria* and *Curcuma xanthorrhiza* using test set

Parameters	Class	Pred (class)	F(CL)	F(Fake)	P(CL)	P(Fake)
% C. zedoaria						
0	CL	CL	1.0558	-0.0558	0.7524	0.2476
10	Fake	Fake	-0.0170	1.0170	0.2623	0.7377
20	Fake	Fake	0.0453	0.9547	0.2871	0.7129
40	Fake	Fake	0.0430	0.9570	0.2862	0.7138
60	Fake	Fake	0.0159	0.9841	0.2753	0.7247
80	Fake	Fake	0.0280	0.9720	0.2801	0.7199
100	Fake	Fake	-0.0410	1.0410	0.2531	0.7469
% C. xanthorrhiza						
0	CL	CL	0.9107	0.0893	0.6945	0.3055
10	Fake	Fake	-0.0515	1.0515	0.2492	0.7508
20	Fake	Fake	-0.0453	1.0453	0.2515	0.7485
40	Fake	Fake	0.0639	0.9361	0.2948	0.7052
60	Fake	Fake	0.0571	0.9429	0.2920	0.7080
80	Fake	Fake	0.0176	0.9824	0.2759	0.7241
100	Fake	Fake	0.0083	0.9917	0.2722	0.7278

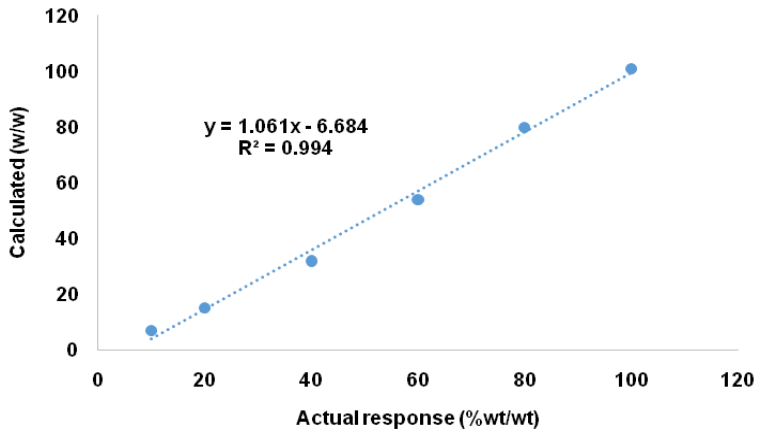

Fig. 6: PLS response plot of the actual and predicted concentration of *Curcuma zedoaria* in a binary mixture with *Curcuma longa*

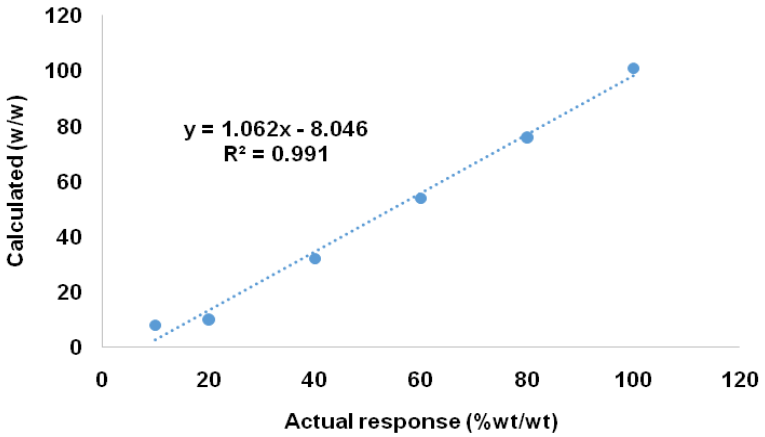

Fig. 7: PLS response plot of the actual and predicted concentration of *Curcuma xanthorrhiza* in a binary mixture with *Curcuma longa*

CONCLUSION

FTIR-ATR spectroscopy combined with chemometrics is a rapid, simple, non-destructive, and inexpensive analytical method for detecting the presence of *Curcuma zedoaria* and *Curcuma xanthorrhiza* in turmeric powder. The different IR spectral pattern between *Curcuma longa*, *Curcuma zedoaria*, and *Curcuma xanthorrhiza* is at frequency region of 1630-1200 cm^{-1}. PCA can discriminate these three species and the adulterated turmeric powder by *Curcuma zedoaria* and *Curcuma xanthorrhiza*. PLS-DA calibration model can classify pure turmeric powder and adulterated turmeric powder accurately. The PLS-R calibration model is valid to quantitate the content of *Curcuma zedoaria* and *Curcuma xanthorrhiza* in adulterated turmeric powder sample.

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AUTHORS CONTRIBUTIONS

EA performed research activity, compiled data, and prepared manuscript. RAS and AR designed research activities, prepared manuscript and made critical thinking on the manuscript.

CONFLICT OF INTERESTS

The authors have declared "no conflicts of interest with respect to the research, authorship, and/or publication of this article".

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