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Analysis of Curcumin contents in *Curcuma xanthorrhiza* using FTIR spectroscopy in combination with Multivariate calibration

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ABSTRACT

Curcuma xanthorrhiza Roxb. or Java Turmeric with the local name of Temulawak is one of herbal medicines used in Indonesia. This plant is believed to prevent some degenerative diseases due to its active compounds, especially curcumin (CUR) contained in Java Turmeric. Therefore, analysis of active components including CUR is very urgent. This study highlighted the development of FTIR spectroscopy coupled with PLSR for the determination of CUR in Java Turmeric powders. The levels of CUR in Java Turmeric powders were determined using HPLC with UV detectors, and the obtained results were used as actual values to be predicted using FTIR spectroscopy-multivariate calibrations. The results revealed that the levels of CUR ranged from 0.6741 ± 0.0705% (g/100 samples) to 2.1062 ± 0.0095%. PLSR modeling for the relationship between the actual value of CUR as determined using HPLC and calculated values as predicted using FTIR spectroscopy provide the value of R^2 of 0.9990 with RMSEC of 0.0028. The developed method offers reliable results providing a green analytical method due to the use of minimum solvent and reagent and does not involve extensive sample preparation.

Keywords: curcumin; PLS regression; HPLC; FTIR spectroscopy; chemometrics.

INTRODUCTION

Curcuma xanthorrhiza Roxb. or Java Turmeric with the local name of *Temulawak* is one of the components typically used in Indonesian herbal medicine. Java Turmeric (JT) is widely distributed throughout Southeast Asia including Indonesia (Kusumadewi et al., 2022). This plant has been reported to have some pharmacological effects including anti-inflammation (Xiang et al., 2018), preventing cancer (Noori et al., 2022), anti-microbial (Septama et al., 2022), methicillin-resistant Staphylococcus aureus (MRSA) infections (Batista de Andrade Neto et al., 2021) and antioxidant activities (Akarchariya et al., 2017). The active compounds reported to be responsible for these activities are xanthorrhizol and curcuminoids (Rohman et al., 2020a). Among curcuminoids, curcumin is more dominant than two others (demethoxycurcumin and bis-demethoxsycurcumin), therefore, to assure the quality of *C. xanthorrhiza*, it is very essential to determine the levels of curcumin since the levels of curcuminoids could be used as authentication analysis of *C. xanthorrhiza* (Rohman et al., 2020b).

Several analytical techniques have been employed for quantitative analysis of curcumin (CUR) in whole plants, in certain extracts, or pharmaceutical preparations such as emulsion including spectroscopic UV (Sharma et al., 2012), synchronized spectrofluorimetric method (Noori et al., 2022), HPLC with UV detection (Syed et al., 2015), HPLC with photodiode array detection (Rafi et al., 2015), HPLC with electrochemical detection (Long et al., 2014), liquid chromatography-tandem with mass spectrometer and NMR spectroscopy (Gören et al., 2009) (Sorng et al., 2022). These analytical techniques typically involved the extensive step of sample preparation and needed the skillful analyst, therefore, some rapid, practical, and easy analytical techniques based on vibrational spectroscopic methods for analysis have been continuously developed offering analytical results that are comparable to those of reference methods.

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Vibrational spectroscopic techniques are methods based on the interaction between EMR in infrared regions with all components present in herbal medicines to provide the vibrational transitions as represented by vibrational spectra (Li et al., 2020). Among vibrational spectroscopic techniques, FTIR spectroscopy is the most popular technique used for the quality control of herbals due to the analytical responses extracted for certain analytical tasks (Singh et al., 2010). With the advancement of statistical software, specific software called chemometrics is applied during data treatment obtained from FTIR spectroscopic measurement which typically involves large data sets (big data analysis). Chemometrics is an application of statistical and mathematical calculation to treat chemical data with the main purpose of obtaining easily understandable information. Pattern recognition and multivariate calibrations are widely applied for the quality control of herbals. The combination of chemometrics of PLS-DA and FTIR spectroscopy has been successfully applied for the authentication of Curcuma species (*C. longa*, *C. xanthorrhiza* and *Zingiber cassumunar*) (Rohaeti et al., 2015), quantitative analysis of curcuminoids in C. longa extracts (Wulandari et al., 2018), and for analysis of curcuminoids in syrup formulations (Prabaningdyah et al., 2018). To our knowledge, the use of FTIR spectroscopy and HPLC combined with PLSR for analysis of CUR in *C. xanthorrhiza* is limited. Therefore, this study aimed to highlight the application of ATR-FTIR spectroscopy coupled with PLSR for quantification of CUR. The results obtained from FTIR spectroscopy were statistically compared with HPLC.

MATERIALS AND METHODS

Materials

The rhizomes of Java Turmeric from 18 locations with different altitudes around Central Java (Wonosobo, Wonogiri, Temanggung, Semarang, Purworejo, Pati, Magelang, Karanganyar, Boyolali, and Banyumas), Special District of Yogyakarta (Kulonprogo and Mangunan), East Java (Ponorogo, Pacitan and Magetan), and West Java (Sukabumi, Bandung, Bogor). All Java Turmeric rhizomes were authenticated in the Plant systematics laboratory, Medicinal Plant and Traditional Medicine Research and Development Center, Tawangmangu, Central Java. Then, all Java Turmeric rhizomes were dried and subjected to powdering to get rhizome powders.

HPLC Analyis

A-25 mg of CUR was accurately weighed and transferred into a 25 mL volumetric flask, added with 20 mL methanol. This solution was shaken vigorously for 5 min and diluted to volume using methanol. Approximately 100 mg of *C. xanthorrhiza* powder was accurately weighed, added with 20 mL methanol, vigorously shaken, and added diluted to 25.0 mL with methanol. These solutions were filtered using a 0.45 filter. The separation of curcumin was carried out using an X-bridge C-18 column (3.0 x 250 mm with particle size 5 µm. Aquadest: acetic acid 2%: acetonitrile (49: 1: 50) was used as mobile phase delivered isocratically with a flow rate of 0.5 mL/min at 1.50 kPa. The analytes were detected using UV at 425 nm with an injection volume of 1 µL. The levels of CUR obtained using HPLC were used as actual values during modeling with FTIR spectra assisted by PLSR.

The acquisition of ATR-FTIR spectra

All powders of Java Turmeric rhizomes were subjected to ATR-FTIR spectral acquisition employing an FTIR spectrophotometer from *Perkin Elmer*® (Massachusetts USA). FTIR instrument was controlled with *Spectrum* 3™ FT-*IR* as operating software. ATR-FTIR spectral acquisition was taken with the specification:

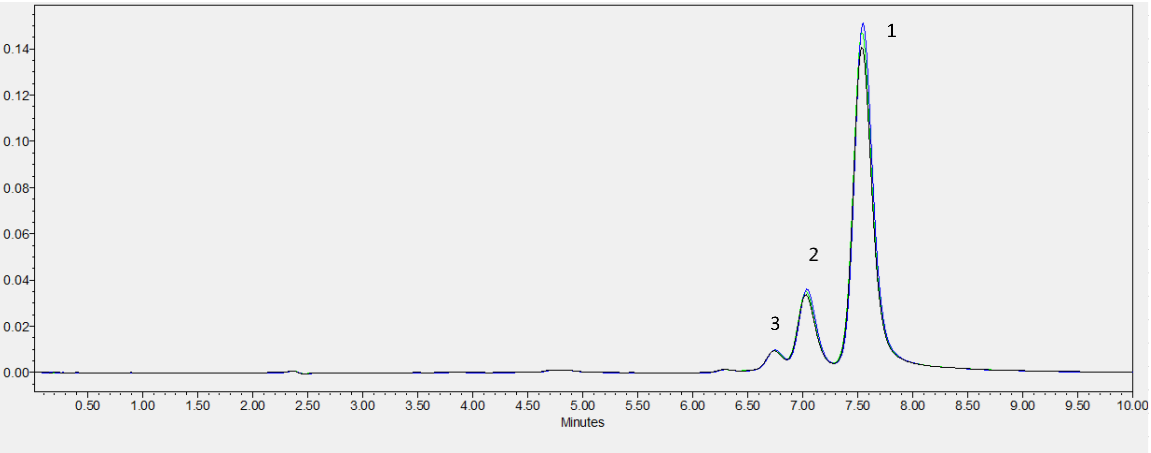
Region : 4000-650 cm^{-1} in mid-infrared
The scanning : 32
Resolution : 8 cm^{-1}

ATR-FTIR spectra of all samples were corrected against the air as background. After every acquisition of ATR-FTIR spectra of samples, a new reference air was measured as background correction. The intensity of ATR-FTIR spectra was scanned as absorbance in three replicates intended for quantitative analysis.

Chemometrics Analysis

The level of CUR in all rhizome samples was expressed as mean (from three replicates) ± standard deviation (SD) with the assistance of Excel software (Microsoft Inc. USA). The absorbances of ATR-FTIR spectra at selected wavenumbers were explored as variables during PLSR. Analysis of multivariate calibration was done using Minitab® version 17.

Figure 1. HPLC separation of curcumin (t_R of near 7.6 min) using column of X-bridge C-18 column (3.0 x 250 mm with particle size 5 µm using mobile phase of Aquadest: acetic acid 2%: acetonitrile (49: 1: 50) delivered isocratic with flow rate of 0.5 mL/min (1: curcumin, 2: desmethoxy curcumin, 3: bidesmethoxy curcumin)



RESULTS AND DISCUSSION

Due to its capability to separate the analyte of interest, curcumin in this study, HPLC using a reversed column was used applying a visible detector at 425 nm. Figure 1 revealed the HPLC chromatogram of curcumin (t_R of nearly 7.6 min) which is separated from other components (tentatively identified as demethoxycurcumin and bis-demethoxsycurcumin) indicating that the HPLC method provides the best separation of curcumin from others. The levels of curcumin in *C. xanthorrhiza* powders from different regions were quantitatively analyzed using HPLC and the results were subjected to ANOVA tests to compare means, as compiled in Table I. The levels of curcumin ranged from 0.6741 ± 0.0705% (g/100 samples) to 2.1062 ± 0.0095%.

ATR-FTIR spectra at 1/λ 4000-650 cm^{-1} are shown in Figure 2. The chemical structure of Curcumin is also included in Figure 2. Each peak and shoulder in ATR-FTIR spectra can be explained by the vibrational transition of functional groups present in the studied Java Turmeric rhizomes. The detailed investigation indicated that the ATR-FTIR spectra of the studied samples are similar since the chemical compositions of all rhizomes are similar. Since ATR-FTIR spectra are fingerprinting in nature, the samples could be differentiated by looking at the absorbance values of peaks and shoulders and the exact wavenumbers of each peak and shoulder. The peak at 1/λ 3300 cm^{-1} appeared broad band indicating the presence of hydrogen bonding due to the stretching vibration of -OH. The alkyl groups of methyl (CH_3) and methylene (CH_2) were observed at 1/λ 2960 cm^{-1} (asymmetric stretching of CH_3), 2922 cm^{-1} (asymmetric stretching of CH_2), and 2875 (asymmetric stretching of CH_2). The strong peaks at 1/λ 1743 cm^{-1} and 1660 cm^{-1} corresponded to unconjugated and conjugated carbonyl (C=O), respectively, while the peak at 1/λ 1512 cm^{-1} is due to the absorption of C=C in stretching vibration mode. Peaks at 1/λ 1454 cm^{-1} and 1372 cm^{-1} could be attributed to CH_2 and CH_3 group vibrations in bending mode. Peaks at 1/λ 1260, 1117, 1097, and 1030 cm^{-1} are due to the stretching vibration of C–O. The peak at 1/λ 960 cm^{-1} corresponds to the stretching vibration of C–OH, while peaks at 1/λ 870 and 760 cm^{-1} originate from functional groups of –HC=CH– (*trans*) and –HC=CH– (*cis*) out of a plane, respectively (Prabaningdyah et al., 2018; Siregar et al., 2018).

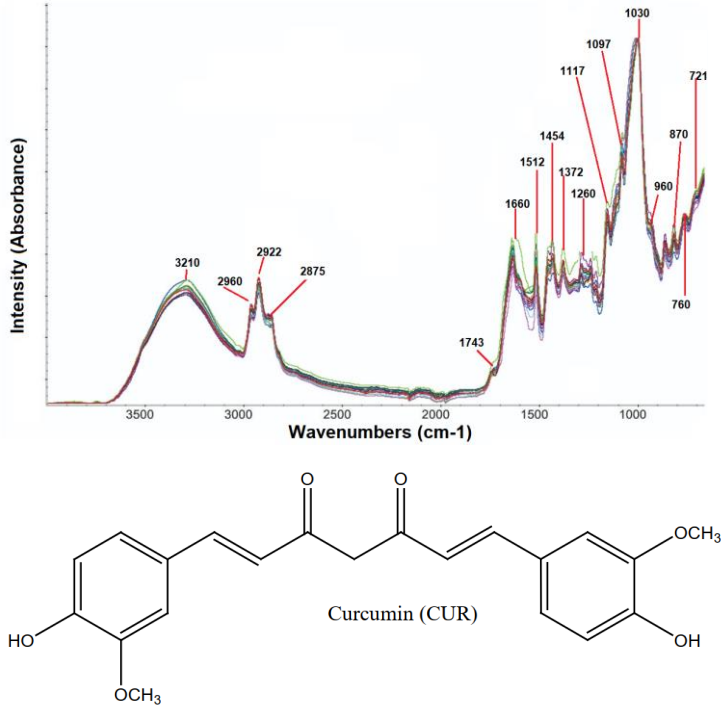
PLSR was used to treat the absorbance values as variables of FTIR spectra at different wavenumbers. PLSR has advantages due to its ability to handle more descriptor variables (absorbance values) of targeted analytes, and to provide non-orthogonal descriptors and multiple responses. In addition, PLSR offers good predictive accuracy with a lower risk of correlation chance. The limitations of PLSR relate to the high risk of overfitting (Cramer, 1993). PLSR is a full-spectrum and factor analysis based on inverse calibration which is very effective for the correlation of two or more variables (Khajehsharifi et al., 2017). Some spectral regions were evaluated for this task, and the selection of wavenumbers regions was based on the capability of the model to provide the best correlation between actual values of curcumin as determined by HPLC and FTIR predicted values,

Table I. HPLC results on curcumin contents of Java Turmeric obtained from different regions.

Origins	Curcumin content (%)
Sukabumi	2.1062 ± 0.0095[a]
Banyumas	2.0279 ± 0.0298[a]
Bandung	1.3706 ± 0.0342[b]
Karanganyar	1.3103 ± 0.0220[ab]
Mangunan	1.2308 ± 0.0034[c]
Boyolali	1.1202 ± 0.0060[d]
Pacitan	1.1096 ± 0.0139[d]
Purworejo	0.9549 ± 0.0143[e]
Ponorogo	0.9388 ± 0.0055[ef]
Kulonprogo	0.8747 ± 0.0343[efg]
Bogor	0.8739 ± 0.0263[fg]
Wonosobo	0.8702 ± 0.0078[fg]
Magetan	0.8432 ± 0.0192[g]
Wonogiri	0.8300 ± 0.0148[g]
Pati	0.8013 ± 0.0146[gh]
Temanggung	0.7423 ± 0.0338[hi]
Semarang	0.7159 ± 0.0216[i]
Magelang	0.6741 ± 0.0705[i]

Means with different lowercase letters within a column in each origin is significantly different ($p<0.05$). Values are means ± SD of triplicate

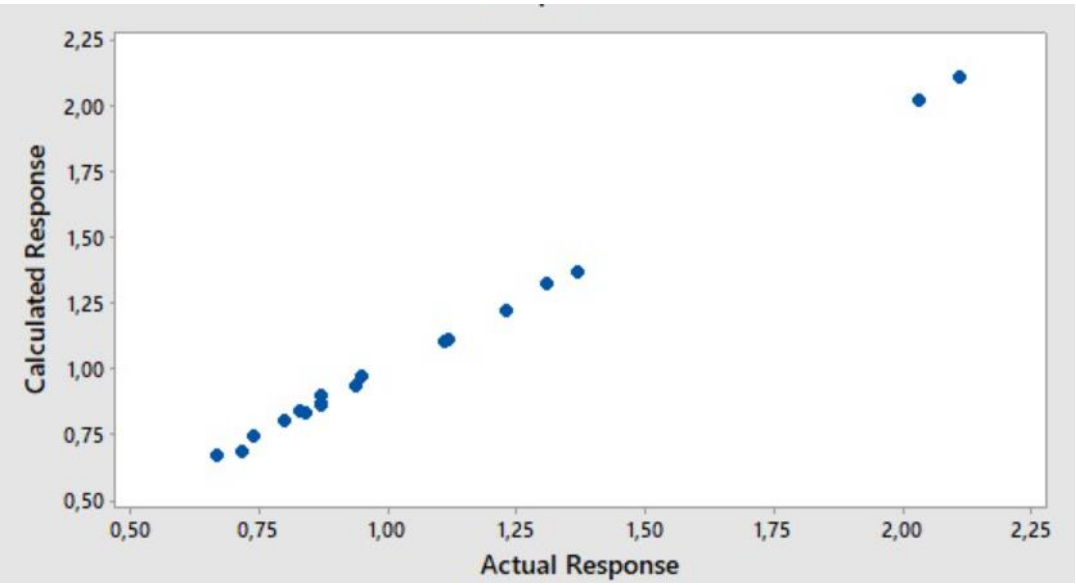
Figure 2. Attenuated Total reflectance-Fourier transform infrared (ATR-FTIR) spectra of *Curcuma xanthorrhiza* powders scanned at 4000-650 cm^{-1} [A] along with the chemical structure of Curcumin [B]



expressed by highest values of R^2 and the lowest values of RMSEC and RMSEP. Finally, the absorbance values at 43 wavenumbers were applied to predict the contents of curcumin.

PLSR modeling for the relationship between the actual value of curcumin as determined using HPLC and the calculated value as predicted using FTIR spectroscopy is depicted in Figure 3. The

Figure 3. Partial least square regression (PLSR) modelling for the relationship between actual value of curcumin as determined using HPLC and calculated value as predicted using FTIR spectroscopy.



values of R^2 were 0.9990 with RMSEC and RMSEP values of 0.0028 and 0.0032. The high value of R^2 and low values of RMSEC and RMSEP indicated that the combination of FTIR spectroscopy and PLSR provides an accurate and precise method for the analysis of curcumin. The similar values of RMSEC and RMSEP also indicated that over-fitting is not observed during calibration and validation modelings (van Wyngaard et al., 2021). FTIR spectroscopy in combination with PLSR offers a direct method for analysis of CUR with simple operational and free from the use of organic solvents since the sample can be directed into ATR sampling accessory. In the future, the combination of FTIR spectra-PLSR can be used as an alternative method for analysis of CUR in *C. xanthorrhiza* powder using HPLC by correlating the peaks and the predicted values obtained during PLSR modeling. In addition, the developed method offers a green analytical method due to the use of minimum solvent and reagent and does not involve extensive sample preparation.

CONCLUSION

FTIR spectroscopy in combination with PLSR is successfully applied for predicting the levels of curcumin in Java Turmeric powder. The relationship between the actual value and FTIR predicted values provides a high value of R^2 and a low value of RMSEC indicating that the developed method is an accurate and precise analytical method for analysis of curcumin. FTIR spectroscopy is rapid and reliable for the analysis of curcumin, providing the green analytical method without any sample preparation.

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