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Analysis of Curcumin in *Curcuma longa* and *Curcuma xanthorriza* Using FTIR Spectroscopy and Chemometrics

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ABSTRACT

FTIR spectroscopy in combination with multivariate calibration of Partial Least Square (PLS) has been developed for quantification of curcumin in the ethanolic extracts of *Curcuma longa* Linn and *Curcuma xanthorriza* Roxb. The optimization was done by selecting the best wavenumbers regions capable of providing the high coefficient of determination (R^2) and low values of Root Mean Square Error of Calibration (RMSEC). Finally, wavenumbers region of 2000-950 cm^{-1} was selected for prediction of curcumin in the extracts. 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. xanthorriza* at 2000-950 cm^{-1} revealed R^2 values of 0.96 and 0.99, respectively. The RMSEC values obtained are 0.299 and 0.089 for *C. longa* and *C. xanthorriza*, respectively. The high value of R^2 and low value of RMSEC indicated the high accuracy and precision of FTIR spectroscopy for quantification of curcumin in the extracts. These results indicated that FTIR spectroscopy combined with PLS is an alternative technique for determination of curcumin in *Curcuma* species. The developed method (FTIR spectroscopy) is rapid, no sample preparation and not involving excessive solvents and reagents.

Key words: Curcumin, FTIR spectroscopy, partial least square, *Curcuma longa* Linn, *Curcuma xanthorriza* Roxb.

INTRODUCTION

The popularity of herbal medicine has risen, not only in Indonesia having the second largest biodiversity after Brazil but also in the worldwide (Zhang *et al*., 2012). *Curcuma longa* known as turmeric is a perennial native plant, where its rhizome is used as a spice, a pigment dye of textiles and in traditional medicine (Jain *et al*., 2007; Rohman, 2012a). *Curcuma longa* is one of the plants from Zingiberaceae family and widely cultivated in the regions of tropical and subtropical, especially in India, South East Asia and China. India is the main country exporting the turmeric and its production is approximately 80%. Today, the species cultivation has also widely distributed to some African countries (Parthasarathy *et al*., 2008). While, *Curcuma xanthorriza*, known as temu lawak in Indonesian community, is an important and potential medicinal plant belonging to the family Zingiberaceae and shares the same genus as *Curcuma longa* Linn (Ab Halim *et al*., 2012).

The main compounds contributing to the activities of *C. longa* Linn and *C. xanthorriza* Roxb. are curcuminoids, including curcumin, demethoxycurcumin and bis-demethoxycurcumin (Fig. 1). Among these, curcumin is present as major component in *C. longa* and *C. xanthorriza*. There are

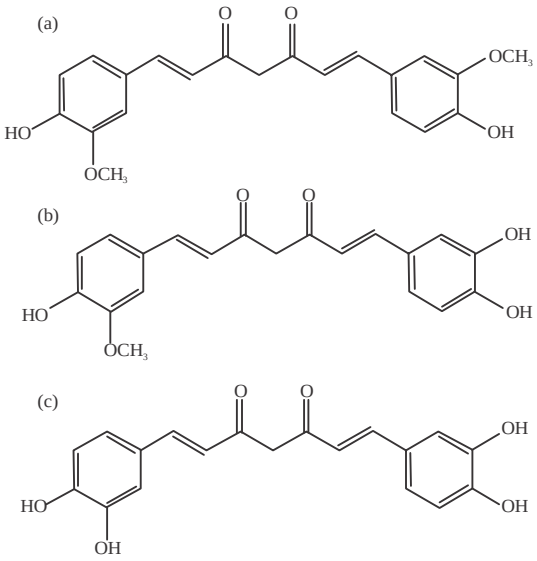

Fig. 1(a-c): Chemical structure of, (a) Curcumin, (b) Demethoxycurcumin and (c) Bis-demethoxycurcumin

several reports regarding the pharmacological activities of curcumin, such as its anti-inflammatory, antimicrobial, antioxidant, antiparasitic, antimutagenic and anticancer properties (Kalpravidh *et al*., 2010; Skrzypczak-Jankun *et al*., 2000; Singh *et al*., 2002; Aggarwal *et al*., 2003). It is also efficient in the treatment of liver diseases and dermatological disorders (Semwal *et al*., 1997).

Some analytical methods, including High Performance Liquid Chromatography (HPLC) and its coupling to mass spectrometry (LC-MS) (Bos *et al*., 2007; Jiang *et al*., 2006), Thin Layer Chromatography (TLC) (Phattanawasin *et al*., 2009; Paramasivam *et al*., 2009) and capillary electrophoresis (Lechtenberg *et al*., 2004) have been used to analyze the curcuminoids in various turmeric samples. However, such chromatographic methods are time-consuming, require experienced personnel to perform the analysis and are destructive. Therefore, some nondestructive and reliable techniques based on vibrational spectroscopy have been developed for quantitative analysis such as near infrared spectroscopy and UV spectrophotometer (Tanaka *et al*., 2008; Pothitirat and Gritsanapan, 2006).

Fourier Transform Infrared (FTIR) spectroscopy, especially in combination with chemometrics technique, has been widely used in analysis of herbal medicine (Rohman *et al*., 2014). The method allows fast, easein sample preparation and non destructive technique. In addition, FTIR spectroscopy can be exploited for determination of components on interesting herbal medicine simultaneously. Using literature review, there is no report regarding the use of FTIR spectroscopy for quantification of curcumin in *C. longa* and *C. xanthorriza*, therefore, this present study aimed to evaluate the application of FTIR spectroscopy in quantitative analysis of curcumin in ethanolic extracts of *C. longa* and *C. xanthorriza*.

MATERIALS AND METHODS

The standard of curcumin was obtained from synthesis and kindly given by Prof. Dr. Sudibyo Martono from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Indonesia. The curcuminoids, i.e., the mixture of curcumin, demethoxycurcumin and

bis-demethoxycurcumin were obtained from Sigma (Alldrich, St. Louis, USA) with the purity of >99%. Acetonitrile and methanol used were of HPLC grade. KBr used for FTIR spectroscopy sampling preparation was purchased from Sigma (Alldrich, St. Louis, USA).

Plant materials and extraction: The rhizomes of *C. longa* and *C. xanthorriza* were obtained from several traditional markets in central java and Daerah Istimewa Yogyakarta, Indonesia in September-October 2013. All rhizome samples were cleaned, cut and air-dried and finally powdered. The powder of *C. longa* and *C. xanthorriza* was subjected to extraction using maceration technique using ethanol as solvent to obtain ethanolic extract of *C. longa* and *C. xanthorriza,* respectively. These extracts were used for FTIR spectroscopic measurement.

FTIR spectral analysis: A 50 mg ethanolic extract of *C. longa and C. xanthorriza* was added with 950 mg KBr IR grade and grinded in mortar until homogen. The mixture was placed on Horizontal Attenuated Total Reflectance (HATR) accessory at controlled ambient temperature (20°C). The FTIR spectra of all samples were scanned using a FTIR spectrophotometer ABB MB3000 (Clairet Scientific, Northampton, UK), equipped with deuterated triglycine sulphate (DTGS) detector and beam splitter of germanium. Using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada) included in the instrument, FTIR spectra were scanned in the mid infrared region of 4000-650 cm^{-1} with resolution of 4 cm^{-1} and number of scanning of 32. The samples were placed in good contact with HATR accessory. All spectra were rationed against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

Quantitation of curcuminoids by High Performance Liquid Chromatography (HPLC): The contents of curcumin in ethanolic extracts of *C. longa* and *C. xanthorriza* determined by HPLC were used as actual value or reference value for those obtained with FTIR spectroscopy in combination with multivariate calibration of Partial Least Square (PLS). The extract was dissolved in mobile phase and an aliquot of 2.0 mL is taken, filtered through an HPLC filter and placed in an autosampler vial of 2 mL. The HPLC analysis was performed using Waters HPLC system (Waters Corp., USA), consisting pump (Waters), ACQUITY solvent manager, waters alliance column heater, Vial Amber Glass 12×32 mm 2 mL with Cap and PTFE/silicone Septum, waters 2767 sample manager and operating software of Empower Basic 2 (Waters, USA). Separation of curcuminoids is performed using C_{18} WatersXterra MS C18 (5 μm; 4, 6×250 mm). The mobile phase used consisted of aquabidestilata and acetonitrile (65:35 v/v) containing 1% acetic acid. The analyte detection was done using UV-vis detector set at λ 425 nm. The injection volume was 20 μL and running time was 40 min.

Statistical analysis: The multivariate calibration of PLS for the correlation between actual value of curcumin as determined with HPLC and FTIR predicted value was performed using Horizon software included in FTIR spectrophotometer. The leave-one-out cross-validation procedure was used to verify the calibration model. The values of Root Mean Square Error of Calibration (RMSEC) and coefficient of determination (R^2) were used as the validity criteria for calibration model. The predictive ability of PLS calibration model was further used to calculate the validation or prediction samples.

RESULTS AND DISCUSSION

Quantification of curcumin by HPLC: Curcumin in ethanolic extracts of *C. longa* and *C. xanthorriza* was initially determined using high performance liquid chromatography with UV-vis detector. Figure 2 exhibited the example of HPLC chromatogram during separation of curcumin from other two curcuminoids using condition as above, either in curcuminoids obtained from Mercck or in ethanolic extract of *C. xanthorriza*. The retention times are 28.191, 31.757 and 35.688 min for bisdes methoxycurcumin, desmethoxycurcumin and curcumin, respectively. *Curcuma xanthorriza* contained curcumin and bidesmethoxycurcumin, as represented by peaks having retention times close to curcumin and bisdesmethoxycurcumin.

Table 1 compiled the levels of curcumin in some ethanolic extract of *C. longa*. While, the level of curcumin in ethanolic extract of *C. xanthorriza* was compiled in Table 2. The levels of curcumin in the evaluated samples are diverse due to the different region, age of plants, etc. Comparing Table 2 and 3, it is known that curcumin concentration in *C. longa* is higher than that in *C. xanthorriza*, as reported by several investigators (Lechtenberg *et al*., 2004; Bos *et al*., 2007; Li *et al*., 2011). The level of curcumin in ethanolic extract of *C. longa* varied from

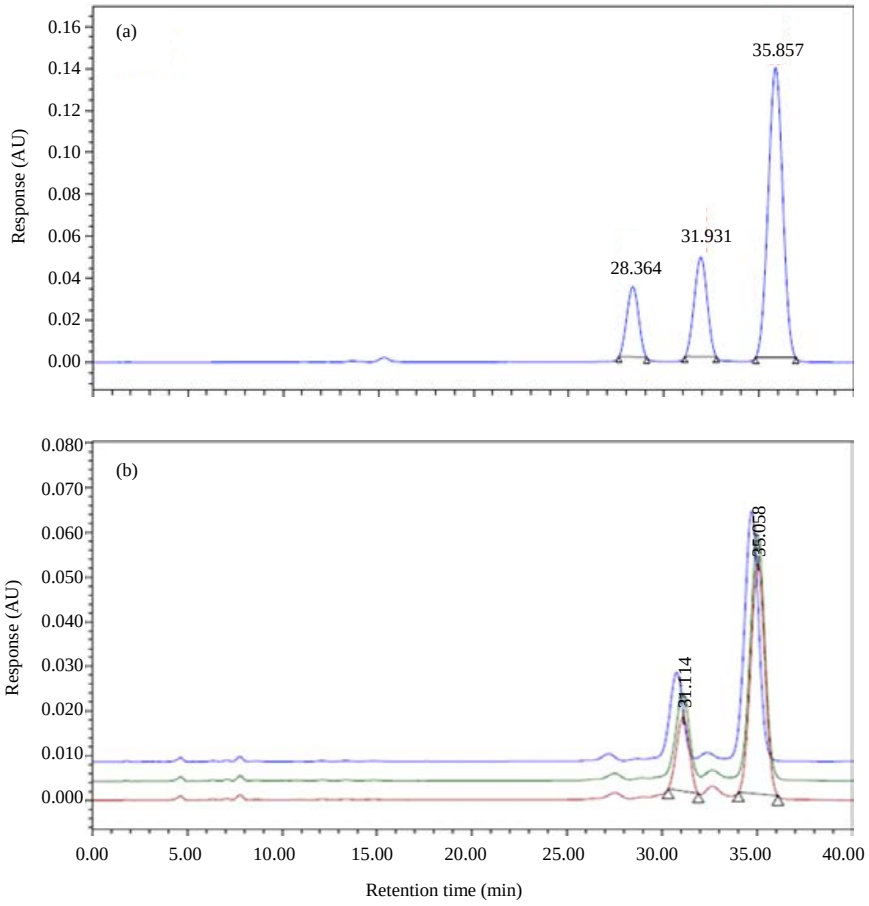

Fig. 2(a-b): The HPLC chromatogram of curcuminoids from (a) E. Merck and (b) Ethanolic extract of *Curcuma xanthorriza*. The retention times for bis desmethoxycurcumin, desmethoxycurcumin and curcumin are 28.191, 31.757 and 35.688 min, respectively.

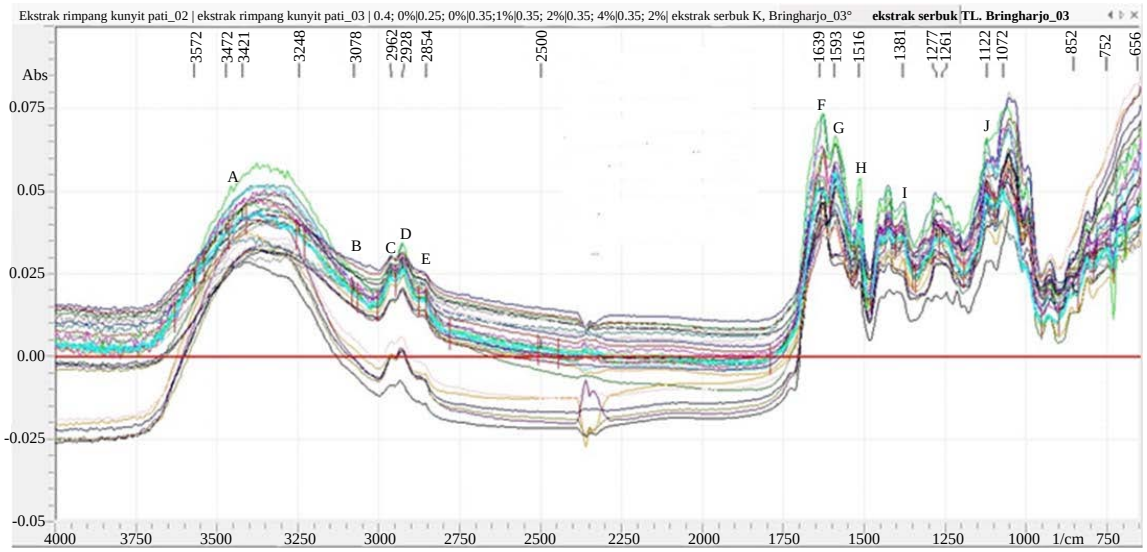

Fig. 3: The FTIR spectra of ethanolic extract of *Curcuma longa* L. scanned at mid infrared region (4000-6500 cm^{-1}). X-axis: Wavenumbers and Y-axis: Response (Absorbance)

Table 1: Levels of curcumin in ethanolic extract of *Curcuma longa* in some regions

Regions	Concentration of curcumin (wt/wt±SD %)
Bantul	5.21±0.43
Magelang	4.78±2.25
Kranggan	7.36±0.09
Godean	5.51±0.13
Purworejo	7.22±0.18
Pati	5.65±0.04
Kudus	4.83±0.14
Boyolali	5.49±0.22

Table 2: Levels of curcumin in ethanolic extract of *Curcuma xanthorriza* in some regions

Regions	Concentration of curcumin (wt/wt±SD %)
Bantul I	2.95±0.01
Godean	2.86±0.03
Bringharjo	2.11±0.04
Purworejo	1.66±0.01
Bantul II	2.97±0.05

4.78±2.25-7.36±0.09% wt/wt, while ethanolic extract of *C. xanthorriza* has curcumin level of 1.66±0.01-2.97±0.05% wt/wt. These values were further used as actual values during correlation with values of curcumin levels obtained using FTIR spectroscopy and multivariate calibration of PLS.

Analysis of curcumin using FTIR spectroscopy and multivariate calibration: Figure 3 revealed FTIR spectra of ethanolic extract of *C. longa* (turmeric) from different regions. Each peak and shoulders come from the absorption of functional groups in *C. longa* extract. Investigation of FTIR spectra of all ethanolic extract of *C. longa* clearly shows the similar peaks due to the similar chemical components contained. However, using detail scrutiny, there is a bit difference in terms of peak intensity (absorbance) caused by different concentrations of components present in ethanolic extract of *C. longa*. The main components of ethanolic extract of *C. longa* is curcuminoids

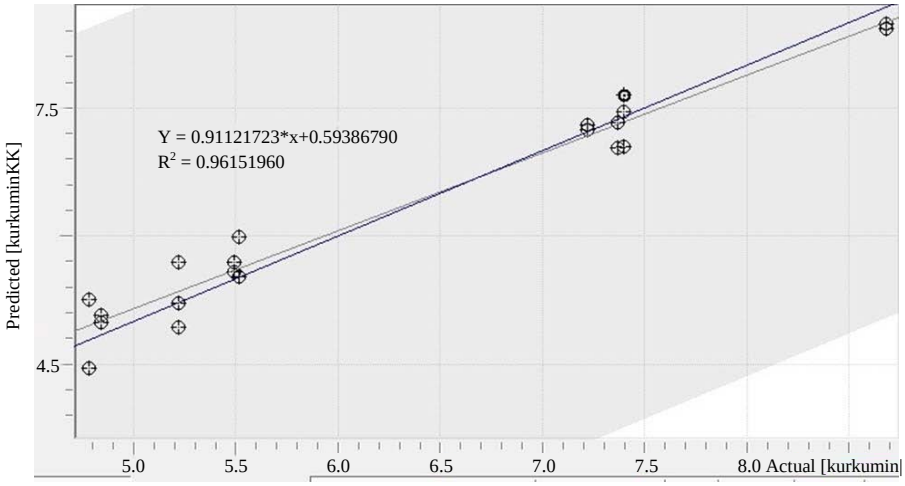

Fig. 4: Correlation between actual values of curcumin in ethanolic extract of *Curcuma longa* determined by HPLC method (x-axis) and predicted values using FTIR spectroscopy combined with PLS (y-axis) at wavenumbers of 2000-950 cm^{-1}

Table 3: Functional groups responsible for infrared absorption of ethanolic extract of *Curcuma longa*

Assignment	Wave numbers (cm^{-1})	Functional groups
A	3371	-O-H stretching vibration
B	3050	C-H aromatic stretching vibration
C	2959	-CH_3-asymmetric stretching
D	2920	-CH_2 asymmetric stretching
E	1624	C = O stretching
F	1589	C = C aromatic stretching
G	1516	Benzene ring bending vibration
H	1423	CH_2 bending
I	1377	CH_3 bending
J	1122	C-O stretching

which refer to phenolic compounds responsible for yellow color in turmeric. As consequence, there is no surprising if peaks coming from functional groups of curcuminoids dominate FTIR spectra of ethanolic extract of turmeric. The functional groups responsible for infrared absorption of ethanolic extract of *C. longa* was compiled in Table 3.

The first step for analysis of curcumin in *C. longa* and *C. xanthorriza* using FTIR spectroscopy combined with multivariate calibration of Partial Least Square (PLS) is the selection of wavenumbers region. The use of FTIR spectra at selected regions can increase the accuracy of analytical results (Vazquez *et al*., 2000). The selection of wave numbers region is based on its capability to provide the high coefficient of determination (R^2) and low values of errors, either in calibration model known as Root Mean Square Error of Calibration (RMSEC) or in prediction model called with Root Mean Square Error of Prediction (RMSEP) (Rohman, 2012b). After optimization step, finally, wave numbers region of 2000-950 cm^{-1} was selected for prediction of curcumin.

Figure 4 and 5 reveled the correlation between actual values of curcumin determined by HPLC and FTIR predicted values using FTIR spectroscopy combined with PLS at wavenumbers of 2000-950 cm^{-1} in ethanolic extract of *C. longa* and *C. xanthorriza*. The R^2 values obtained are 0.96 and 0.99 in *C. longa* and *C. xanthorriza*, respectively. The RMSEC values obtained are 0.299 and 0.089. The high value of R^2 and low value of RMSEC indicated the high accuracy and precision of analytical method.

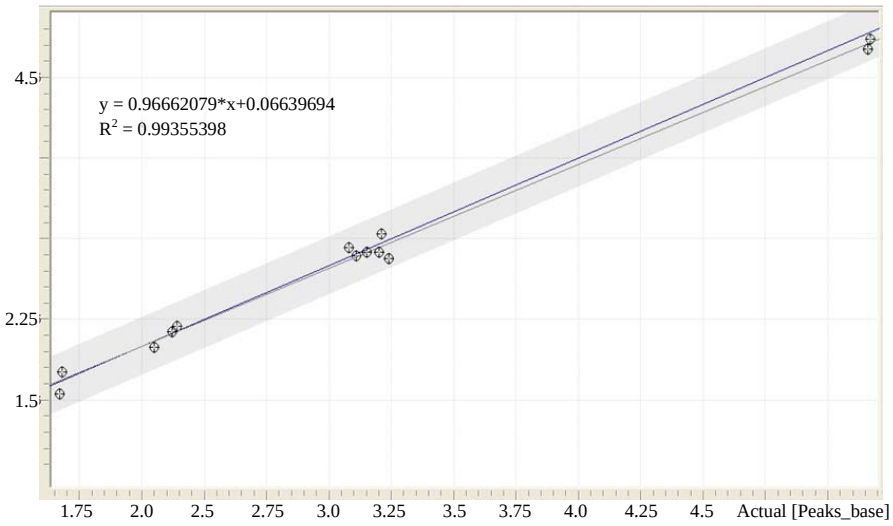

Fig. 5: Correlation between actual values of curcumin in ethanolic extract of *C.* xanthorriza determined by HPLC method (x-axis) and predicted values using FTIR spectroscopy combined with PLS (y-axis) at wavenumbers of 2000-950 cm^{-1}

CONCLUSION

These results indicated that FTIR spectroscopy combined with PLS is an alternative technique for determination of curcumin in *Curcuma* species.

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