Application of FTIR spectroscopy and chemometrics for correlation of antioxidant activities, phenolics and flavonoid contents of Indonesian Curcuma xanthorrhiza

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ABSTRACT

Curcuma xanthorrhiza is one of the most commonly used herhals found in Indonesian Traditional Medicine. This herb has been known as antioxidant. The phenolics and flavonoids are believed to be responsible for these activities. The objective of this study was to develop FTIR (Fourier transform infrared) spectra in conjunction with chemometrics of multivariate calibration for the prediction of antioxidant activity, total phenolics contents (TPC) and total flavonoid contents (TFC) of \textit{C. xanthorrhiza} obtained from several locations in Indonesia. In this study, \textit{C. xanthorrhiza} samples were subjected to antioxidant activity measurement based on DPPH radical scavenging assay and expressed by inhibition concentration of 50\% (IC\textsubscript{50}), determination of TPC based on Folin-Ciocalteau reagent expressed as gallic acid equivalent (mg GAE/g) and determination of TFC based on its complexes with AlCl\textsubscript{3} and expressed as quercetin equivalent (mg QE/g). The samples were also scanned using FTIR spectrophotometer at wavenumbers of 4000–650 cm\textsuperscript{-1} based on attenuated total reflectance (ATR) measurement. The antioxidant activities, TPC and TFC were correlated with FTIR spectra with the aid of multivariate calibration. The results showed that IC\textsubscript{50} values of \textit{C. xanthorrhiza} powder were within 0.177–0.615 mg/mL, TPC of 0.438–1.214 mg GAE/g and TFC of 0.058–0.229 mg QE/g. PLS regression for the correlation between actual values of IC50 and FTIR predicted values were of 0.9919 indicating the high accurate prediction method. From ANOVA test, the error obtained is low, i.e., 0.0027 indicating the precise method. The similar results in terms of high accuracy and precision were also obtained during the employing FTIR spectra and PLS for prediction of TPC and TFC. It can be concluded that the combination of FTIR spectroscopy and chemometrics can provide the alternative methods for prediction of antioxidant activities using DPPH radical assay, TPC and TFC with the main advantage of simple in operation and minimum use of solvents.

Introduction

Curcuma xanthorrhiza, also known as Temulawak or Javanese turmeric, belongs to Zingiberaceae family and cultivated around the world, especially in Southeast Asia like Indonesia and Malaysia. This plant is one of herbal components used as medicinal plant in Indonesia. \textit{C. xanthorrhiza} is reported to have some biological activities to cure certain diseases and to promote the human health \cite{1}. Traditionally,
C. xanthorrhiza is utilized as home remedies, such as *Jamu* prescriptions, food supplements, and herbal drinks. The biological activities reported in *C. xanthorrhiza* are antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-candida, anti-hyperglycemic, and antihypertensive effects. Among these pharmacological activities, the studies on antioxidant activities are extensively reported either in *vitro* or in *vivo*. Some bioactive compounds such as xanthorrhizol, curcuminoids (curcumin, desmethoxycurcumin), flavonoids, camphor, phelandrene, tumerol, borneol, sineol and sesquiterpenes.

With the issues related to some restrictions of synthetic antioxidant, in the recent years, the exploration of natural antioxidant is carried out massively among scientist to get effective and safe antioxidants to human health. Some definitions of antioxidants existed, and one of them are any materials, phytochemicals or chemical compounds either natural or synthetic capable of delaying or preventing significantly the oxidation reactions in the oxidized models at low concentrations. C-1: References should be number format? Curcuma species such as *C. longa*, *C. xanthorrhiza*, *C. manga* and *Curcuma aeruginosa* Roxb. are reported to have strong antioxidant activities through studies of in *vitro* and *vivo* due to curcuminoids contained in those plants.

Several methods are proposed to be used during the investigation of the antioxidant activities of herbal materials either in *vitro* or in *vivo* approaches. The mechanisms underlying the antioxidant measurements include radical scavenging activities using some radicals (ABTS, DPPH; nitric oxide, hydroxyl, etc.), lipid peroxidation inhibition (such as linoleate-thyocyanate, beta-carotene bleaching), reducing power (ferric reducing activity power or FRAP, Folin-Ciocalteau for reducing agents), chelating agents (cupric ion reducing antioxidant capacity known as CUPRAC) and synergistic effects. Among these methods, DPPH radical scavenging assay is the most popular methods applied for screening the antioxidant activities of herbal. However, all these methods require some preparation steps, time consuming, and using much solvents. Therefore, it is important to develop rapid and efficient analytical methods that support green analytical chemistry the antioxidant activity, total phenolic content, and total flavonoid content of herbal products. The antioxidant activities of herals varied with the growth location, harvesting time and other environmental conditions. In addition, the evaluation of in *vitro* antioxidant activities is typically correlated with phenolics and flavonoid groups, therefore, in this study the correlation between antioxidant activities with phenolics and flavonoid contents was also investigated.

Following the development in chemometrics software, new methods based on rapid instrumental analysis such as vibrational spectroscopy have been proposed to predict the antioxidant activities. FTIR spectroscopy is a fingerprint method that allows for fast analysis with minimum sample preparation and solvent used. The vibration of compounds responsible for antioxidant activities such as phenolics and flavonoid compounds could be measured using FTIR spectroscopy. Therefore, it can be used to predict the antioxidant activities of samples. Irnawati et al. recently have predicted the antioxidant activities of pumpkin seed oil using the combination of FTIR spectra and chemometrics of multivariate calibrations. The combination of UV-Vis spectra, NMR spectra, and fluorescence spectroscopy with chemometrics is also successful for profiling of the evaluated samples based on their correlation with DPPH radical scavenging activities. However, study on the use of FTIR spectroscopy for correlation of antioxidant activities, phenolic content, and flavonoid content is still limited. Moreover, there is no report on the study of antioxidant, phenolic content, and flavonoid content of *C. xanthorrhiza* using FTIR spectroscopy and chemometrics. Therefore, this study was directed to: (i) investigate antioxidant activities, phenolics contents and flavonoid contents in Indonesian *C. xanthorrhiza* contents, (ii) to use the chemometrics techniques for the correlation between antioxidant activities, phenolics contents and flavonoid contents and for classification of *C. xanthorrhiza* according to its origin, and (iii) to predict DPPH radical scavenging activities based on FTIR spectra combined with multivariate calibrations.
Material and methods

Material

Curcuma xanthorrhiza rhizomes were obtained from 18 locations with different altitudes namely Semarang, Banyumas, Boyolali, Karanganyar, Magelang, Purworejo, Temanggung, Wonogiri, Wonosobo, Pati (Central Java), Kulonprogo, Mangunan (Yogyakarta), Magetan, Pacitan, Ponorogo (East Java), and Bogor, Bandung, Sukabumi (West Java). These rhizomes were authenticated in Plant systematics laboratory, Medicinal Plant and Traditional Medicine Research and Development Center, Tawangmangu, Central Java. The rhizomes were cleaned in running water, sliced into small pieces then dried. The dried rhizomes were ground into powder to get rhizome powders. The powdered rhizomes were subjected to several evaluations.

Free radical-scavenging activity using DPPH

DPPH radical-scavenging activity of all evaluated samples was carried out by .[16] Briefly, 1 mL of the sample solution with certain concentration was methanol in 10 mL volumetric flask. For each sample, radical scavenging capacity (RSA) was evaluated by investigating the absorbance values of DPPH• solution 0.4 mM at 517 nm (designated as Abs control) and the absorbance of DPPH• solution added with C. xanthorrhiza samples after the operating time of 30 min (designated Abs sample). All absorbance values were corrected with absorbance of methanol and samples since C. xanthorrhiza samples had absorbance value in 517 nm. Trolox was used as the positive control in this experiment. For the calculation of radical scavenging activity percentage (%), the following formula was used:

\[
\text{Percentage} \text{ (~)} \text{of DPPH RSA} = \left( \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \right) \times 100\%
\]

RSA using DPPH radical is expressed as IC\textsubscript{50} (the concentration of a sample required to decrease 50% absorbance of DPPH radicals). IC\textsubscript{50} value can be depicted graphically by plotting the percentage of RSA of DPPH radicals (y-axis) against the concentration or log concentration of samples (x-axis) to form linear equation .[17]

Total phenolic contents

The levels of total phenolic content (TPC) in C. xanthorrhiza powder were determined based on Folin-Ciocalteau (F-C) .[18] A-0.5 mL of 10% F-C reagent from Sigma (Aldrich, USA), 1.5 mL 7.5% sodium carbonate (E. Merck, Germany) and 7.9 mL distilled water (Ikapharmindo, Indonesia) were introduced into a test tube containing 0.1 g samples. After that, the sample solution was mixed scrupulously and allowed to stand for 1 h in a dark place (based on operating time study). The absorbance of blue-colored solutions was measured at 754 nm by UV–VIS Spectrophotometer (Shimadzu, UVmini-1240). The TPC of C. xanthorrhiza powder was expressed as mg of gallic acid equivalents (mg GAE)/g samples.

Total flavonoid content (TFC)

The levels of total flavonoid content (TFC) were quantitatively analyzed according to a method carried out by .[19] A-0.1 g of powder samples was added with 4 mL distilled water, followed by the addition of 0.5 mL solution of AlCl\textsubscript{3} 2%. The mixture was stood for 30 min. The absorbance was measured at 510 nm using UV–VIS Spectrophotometer (Shimadzu, Japan). All analysis were performed in triplicate. The levels of TFC was calculated as mg of quercetin equivalents (mg QE/ g samples).
**Scanning of FTIR spectra**

The rhizome powders are subjected to FTIR spectral measurement. The spectra were scanned using FTIR spectrophotometer (Perkin Elmer®, Massachusetts USA), controlled with the operating software of Spectrum 3™ FT-IR. The measurements were done in mid-infrared region of 4000–650 cm⁻¹ with scanning number of 32 and the resolution of 8 cm⁻¹. The used sampling accessory was horizontal attenuated total reflectance (HATR) composed of ZnSe crystal. All FTIR spectra were corrected against FTIR spectrum of air as background. After every scan, a new reference air background spectrum was taken. These spectra recorded as absorbance values at each data point in triplicate were used for making the correlation between antiradical activities, phenolics contents and flavonoid contents with FTIR spectra.

**Chemometrics analysis**

The antiradical activities, phenolics contents and flavonoid contents were expressed as mean ± standard deviation (SD) with the aid of Excel software (Microsoft Inc. USA). The absorbance values of FTIR spectra were used as variables during chemometrics analysis. All chemometrics analysis (PCA, CA and PLS) were carried out using Minitab® version 17 (Minitab Inc., USA).

**Results and discussion**

FTIR spectra of powder of Java turmeric scanned at mid-infrared region corresponding to wavenumbers of 4000–650 cm⁻¹ were depicted in Figure 1. Each peak and shoulder in FTIR spectra correspond to stretching and bending vibrations of functional groups which absorb IR radiation resulting the vibrational transitions among bonds. The shape of FTIR spectra among samples is similar, however, using detailed investigation it is obvious that peak intensities among samples are different. This is not surprising because FTIR spectra are characterized as fingerprinting analytical tools for differentiation and characterization of samples or objects including herbal medicines. Peak at 3300 cm⁻¹ (a) originates from stretching vibration of -OH (hydrogen bonding), peak at 2930 cm⁻¹ (b) is due to stretching vibration of methyl and methylene (CH₃, CH₂), peak at 1640 cm⁻¹ is specific for stretching vibration of carbonyl (C = O) conjugated to other double bonds, while double bond of C = C in stretching vibration mode was observed at near 1517 cm⁻¹ (d). The bending vibration of methyl was clearly observed at wavenumbers of 1370 cm⁻¹ (e). The presence of amine group was confirmed by stretching vibration of C-N at 1331 cm⁻¹ (f). Peaks at 1264 (g), 1154 (h) and 1073 (i) corresponded to stretching vibration of C–O. Peak at 996 cm⁻¹ (j) is due to stretching vibration of C–OH, while peaks at 861 (k) and 770 cm⁻¹ (i) are due to – HC = CH – (trans) out of plane and – HC = CH – (cis) out of plane, respectively . The vibrations resulted from FTIR scanning supported the presence of phenolic and flavonoid content in C. xanthorrhiza. These compounds are responsible for antioxidant activities. Therefore, it supports the potential application of FTIR spectroscopy for determining the antioxidant activities, phenolic content and flavonoid content in C. xanthorrhiza. Moreover, the presence of – OH, C–O, C = C, CH₂- and CH₃ support the presence of xanthorrhizol in the evaluated samples, because xanthorrhizol (included in Figure 1) is reported as the main component in Java Turmeric .

Table 1 compiled the analytical results of Java turmeric samples in terms of antioxidant activities (AA) expressed by Inhibition Concentration of 50% DPPH radicals known as IC₅₀ ranging from 0.177 to 0.615 mg/mL, total phenolics contents (TPC) ranging from 0.438 to 1,214 gallic acid equivalent (mg GAE/g) and total flavonoid contents (TFC) ranging from 0.058 to 0.229 quercetin equivalent (mg QE/g). The large variety of antioxidant activities, TPC and TFC is advantageous in terms of multivariate
calibration modeling, therefore all the data were included during modeling. Variations on the antioxidant activities, phenolic content, and flavonoid content were associated to the difference of metabolite compositions of *C. xanthorrhiza* in each region. The multivariate calibration of partial least square (PLS) was used for making the correlation between actual values of AA, TPC and TPC with FTIR predicted values using variable of absorbance values of selected peak regions. The selection of peak wavenumbers was relied on the capability of this region to provide the best accuracy as expressed by high coefficient of determination ($R^2$) and the highest precision as expressed by low value of errors \[22\]. PLS is one of inverse multivariate calibration widely applied for chemometric modeling intended for the prediction of concentrations in the unknown samples using latent variables which are combination of original variables (absorbance values). The acceptability of PLS models was evaluated by some statistical parameters such as $R^2$-calibration, $R^2$ validation, errors in calibration and validation and number of variables \[23\].

The accuracy evaluation of this method was evaluated by coefficient of determination ($R^2$-value), a measure for the close agreement between actual values and measured values. In addition, the precision of the developed method was evaluated using errors during modeling obtained from ANOVA test. Figure 2(a) revealed the plot the correlation of actual values of antioxidant activities (x-axis) and FTIR

Figure 1. Normal FTIR spectra of Java Turmeric powders scanned at wavenumbers of 4000–650 cm$^{-1}$ using attenuated total reflectance (ATR) mode.
Table 1. The antioxidant activities, phenolics contents and flavonoid contents of Indonesian Curcuma xanthorrhiza powders.

| Origins       | Total Flavonoid contents (quercetin equivalent) | Total phenolic contents (gallic acid equivalent) | Antioxidant activity (IC50 in mg/mL) |
|---------------|-----------------------------------------------|-------------------------------------------------|-------------------------------------|
| Karanganyar   | 0.161 ± 0.010<sup>bc</sup>                    | 0.903 ± 0.016<sup>bc</sup>                      | 0.247 ± 0.000<sup>bc</sup>          |
| Semarang      | 0.085 ± 0.012<sup>efg</sup>                    | 0.454 ± 0.032<sup>bc</sup>                      | 0.507 ± 0.002<sup>bc</sup>          |
| Banyumas      | 0.182 ± 0.026<sup>bc</sup>                    | 0.927 ± 0.053<sup>bc</sup>                      | 0.189 ± 0.009<sup>bc</sup>          |
| Wonosobo      | 0.086 ± 0.009<sup>def</sup>                    | 0.557 ± 0.020<sup>de</sup>                      | 0.506 ± 0.005<sup>c</sup>           |
| Wonogiri      | 0.078 ± 0.005<sup>fg</sup>                    | 0.479 ± 0.021<sup>bc</sup>                      | 0.498 ± 0.007<sup>c</sup>           |
| Boyolali      | 0.124 ± 0.010<sup>def</sup>                    | 0.830 ± 0.066<sup>cd</sup>                      | 0.315 ± 0.007<sup>bc</sup>          |
| Purworejo     | 0.078 ± 0.027<sup>fg</sup>                    | 0.552 ± 0.009<sup>bc</sup>                      | 0.447 ± 0.011<sup>d</sup>           |
| Temanggung    | 0.090 ± 0.026<sup>defg</sup>                   | 0.515 ± 0.003<sup>e</sup>                      | 0.592 ± 0.006<sup>d</sup>           |
| Magelang      | 0.065 ± 0.009<sup>g</sup>                    | 0.438 ± 0.008<sup>bc</sup>                      | 0.548 ± 0.006<sup>d</sup>           |
| Magetan       | 0.066 ± 0.003<sup>g</sup>                    | 0.506 ± 0.009<sup>bc</sup>                      | 0.457 ± 0.005<sup>bc</sup>          |
| Pacitan       | 0.134 ± 0.021<sup>bcd</sup>                   | 0.594 ± 0.025<sup>de</sup>                      | 0.328 ± 0.007<sup>bc</sup>          |
| Ponorogo      | 0.128 ± 0.003<sup>cde</sup>                   | 0.706 ± 0.291<sup>cde</sup>                     | 0.404 ± 0.005<sup>e</sup>           |
| Mangunan      | 0.131 ± 0.021<sup>cde</sup>                   | 0.684 ± 0.021<sup>cde</sup>                     | 0.246 ± 0.004<sup>bc</sup>          |
| Bogor         | 0.061 ± 0.008<sup>g</sup>                    | 0.622 ± 0.010<sup>bc</sup>                      | 0.176 ± 0.002<sup>b</sup>           |
| Pati          | 0.058 ± 0.004<sup>g</sup>                    | 0.476 ± 0.032<sup>e</sup>                      | 0.614 ± 0.006<sup>e</sup>           |
| Sukabumi      | 0.229 ± 0.013<sup>x</sup>                    | 1.153 ± 0.010<sup>ab</sup>                      | 0.289 ± 0.005<sup>b</sup>           |
| Bandung       | 0.214 ± 0.019<sup>x</sup>                    | 1.214 ± 0.222<sup>x</sup>                      | 0.230 ± 0.022<sup>e</sup>           |
| Kulonprogo    | 0.084 ± 0.008<sup>efg</sup>                   | 0.556 ± 0.021<sup>de</sup>                      | 0.361 ± 0.005<sup>k</sup>           |

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

predicted values (y-axis) assisted by PLS regression using variable of absorbance values at 97 wave-numbers (Supplementary data 1) providing R<sup>2</sup> value of 0.9919 indicating the high accurate prediction method. From ANOVA test, the error obtained is low, i.e., 0.0027 using 10 variable components (a combination of original 97 variables) indicating the precise method. The PLS model was built using ten

![Figure 2](image-url)
Furthermore, the contribution of each variable during PLS modeling was shown in Figure 2(b) indicating that the used variables contributed significantly in predicting the antioxidant activities of *C. xanthorrhiza*. Therefore, FTIR spectra in combination with PLS could be used as a rapid and efficient method for predicting the antioxidant activities of *C. xanthorrhiza* from different origins.

The same absorbance values extracted from the FTIR spectra were also used as variables during the prediction modeling of TPC and TFC based on FTIR spectra and PLS regression. Figure 3(a) revealed the correlation plot of PLS modeling between actual and calculated responses of TPC providing $R^2$-value of 0.9964 and error of 0.0002 (using 10 variable components), while the plot for relating actual and calculated responses of TPC using PLS regression provide $R^2$-value of 0.9986 and
residual error of 0.0014 as evaluated by ANOVA test. The high value of $R^2$ expressed good of fitness and high accuracy of the PLS model whereas the low error value indicated good precision of PLS model to predict the phenolic content and flavonoid content in C. xanthorrhiza. Based on the results of $R^2$-values and errors during PLS modeling, FTIR spectroscopy in combination with multivariate calibration can be developed as an alternative method to the reference method with main advantages of its simplicity, minimum or without sample preparation step, and minimum use of solvents supporting the use of green analytical method during quality control of herbal preparations.

**Conclusion**

FTIR spectroscopy in combination with multivariate calibration of partial least square (PLS) regression provide accurate and precise method for the prediction of antioxidant activities, total phenolics contents (TPC) and total flavonoid contents (TFC). PLS regressions were successfully applied for modeling the correlation between actual and calculated responses with $R^2$-values of $>0.99$ and errors within $0.0002 – 0.0027$ indicating the acceptable accuracy and precision of the developed method. Furthermore, the proposed method can be used as an alternative method during the quality control of herbal preparations. In the future, the collaborative studies among competent laboratories to use the proposed method are needed.

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**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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