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Application of FTIR Spectroscopy and Chemometrics PLSR Of the Determination of Total Flavonoid of Kalimantan's Kasturi (*Mangifera casturi*).

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ABSTRACT

Fourier transform infrared (FTIR) spectroscopy combined with chemometrics of multivariate calibration of partial least square regression (PLSR) developed for analysis of total flavonoid content determination of Kasturi. PLSR was choosed to developed the prediction total flavonoid content. The calibration model was prepared by making the relationship between actual values of total flavonoid as determined using standard method spectrophotometrically in visible region ($AlCl_3$ method) and FTIR predicted value aid with PLS. The developed calibration model was further validated using independent samples.The coefficient determination (R2) values for such correlation obtained are 0.998 and 0.995 for calibration and validation samples, respectively. The root mean square error of calibration (RMSEC) value of the developed model was 0.205% and the root mean square error of prediction (RMSEP) value was 0.831. Based on these findings, it can be concluded that FTIR spectroscopy combined with PLSR can be used accurately and precisely to predict the total flavonoid content of kasturi with the regression equation of predicted value (y) = 0.979 x actual value as determined by $AlCl_3$ method + 0.065

Keywords: FTIR spectroscopy, partial least square regression, Kasturi, Total Flavonoid,

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INTRODUCTION

Essentially, active compound in plant is a series of organic compounds which have functional groups (Devika et al, 2013). Kasturi (*Mangifera casturi*) is one of the typical plant in South Kalimantan. Sutomo *et al* (2013) states that Kasturi contain flavonoids. Kasturi has been reported to have some biological activities such as antioxidant and antiinflamatory (Sutomo *et al* 2013, Fakhrudin *et al*, 2013)

Quantitative analysis method of active compounds of plants has an important role in the development of medical products using medicinal plants. The current standard method for analysis of total flavonoids is $AlCl_3$ method determined spectrophotometrically in visible region (Desmiaty, 2009). However, this method need special reagents and solvents, therefore, analytical method offering direct method for analysis of total flavonoid content in some extracts must be addresses. One of the developed methods currently proposed by some researchers are Fourier transform infrared (FTIR) spectroscopy, especially if combined with sophisticated chemometrics technique of partial least square (PLS) calibration.

FTIR spectrophotometry is one of the vibrational spectroscopy (Rohman et al., 2014), besides Raman spectroscopy and imaging spectroscopy. This technique allows researchers to perform qualitative and quantitative analysis of active compounds in plants (Stuart, 2004) due to its nature as fingerprint technique. In addition, the chemometrics applied in FTIR spectroscopy will give the development of a multivariate calibration from a simple spectra to the complex spectra (Ma, 2000). In addition, the availability of modern software in chemometric generates a precise data model and more robust calibration model (Ma, 2000).

The application of FTIR spectroscopy in combination with chemometric of multivariate calibration in medical plants and related products has been reported such as for the authentication of Red Fruit Oil (Rohman *et.al*., 2010), analysis of oil parameters of red fruit oil (Triyasmono *et al*., 2013; Andina *et al*., 2014; Rohman et al, 2015); classification of Meniran or *Phylantus niruri* (Dharmaraj *et.al*., 2006), determination the levels of total antioxidant (Versari et.al., 2010), and the assay of total flavonoids of Tempuyung (Rohaeti *et.al*., 2011). Using literature review, there is no report available for analysis of total flavonoid contents in Kasturi. This research developed FTIR spectroscopy in combination with chemometric of PLS to determine the levels of total flavonoid contents in Kasturi. The prediction model of total flavonoids using FTIR spectroscopy-PLS is evaluated in terms of coefficient of determination (R^2), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).

MATERIALS AND METHODS

Kasturi (*Mangifera Casturi*) was obtained from Banjar Baru, West Kalimantan, Indonesia. The authenticity of this plant was carried out in Department of Biology of Lambung Mangkurat University, KBr for IR spectroscopy grade, $AlCl_3$, quercetin pa organic solvents and reagents were obtained from E. Merck (Darmstat, Germany).

Preparation of ethanolic extract of Kasturi

The ethanolic extract of Kasturi was prepared using ethanol 70% as solvent. The plant was dried, and made into powder. The powder is maserated using ethanol 70% for 6 hours. The extract was evaporated in rotary evaporated to obtain the dry extract. The dry extract obtained was used to make ethanolic extract of kasturi with three different concentrations, i.e. 25%, 50% and 70%.

Determination of total flavonoid by UV-Vis Spectroscopy

Determination of total flavonoid contents of ethanolic extract was performed using visible spectroscopy according to Rohaeti *et al*. (2011). The dry extract was used to make solution with concentration of 1000 ppm (prepared by weinging 10 mg extract accurately and then solved in 10 mL ethanol proanalytical grade). A-1.0 mL of extract in a test tube was reacted with 1 mL of $AlCl_3$ 2%. Subsequently, 8 mL of acetic acid 5% was added into the solutions. The sample solutions were left in the dark for 12 minutes. The absorbance of the sample solutions and blank solution were measured on 415 nm using spectrophotometer UV-Vis. The measurements were carried in three times of replication

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Measurement of FTIR spectra

Approximately of 50.0 mg of dry extracts were mixed and homogenized with 950 mg KBr for IR spectroscopy. All samples were measured using FTIR spectrophotometer FTIR MB 2000 and were recorded in 32 seconds with 4 cm^{-1} of resolution. All spectra were subjected to partial least square (PLS) Regression using Horizon MB 2000 software included in FTIR spectrophotometer without derivatization.

Statistical data

Multivariate calibration model for the prediction between actual value of flavonoid content and FTIR spectroscopy was build with partial least square regression. The validation model was evaluated with cross validation technique. The accuracy and the precission of the model can be seen by the coefficient of determination value (R^2), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). The prediction model will be accepted if values of RMSEC and RMSEP are low, and value of coefficient of determination is high (Miller and Miller, 2010).

RESULTS AND DISCUSSION

Determination of Total flavonoid of Kasturi using visible spectroscopy

The principle of the determination of total flavonoid with $AlCl_3$ method (visible spectroscopy) is the formation of a complex between $AlCl_3$ with C-4 keto group and C-3 or C-5 hydroxy group of flavonoid compounds (Desmiaty, 2009). Quercetin was used as a standard for the determination of total flavonoid. Quercetin is a flavonoid from flavonol group having C-4 keto group and C-3 or C-5 hydroxy groups. Fig. 1 shows the reaction of the complex formation between quercetin and $AlCl_3$. Standard curve obtained from the determination of quercetin standard in concentration of 50, 60, 70, 80, 90, 110, 120, 130 and 140 ppm, can be seen in Fig. 2. The standard curve of quercetin was made as an equivalent comparison of flavonoid compounds contained in ethanolic extract of Kasturi, therefore it was used to determine the level of flavonoid total content.

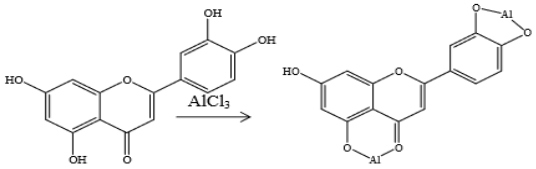

Figure 1. Reaction of complex formation with $AlCl_3$

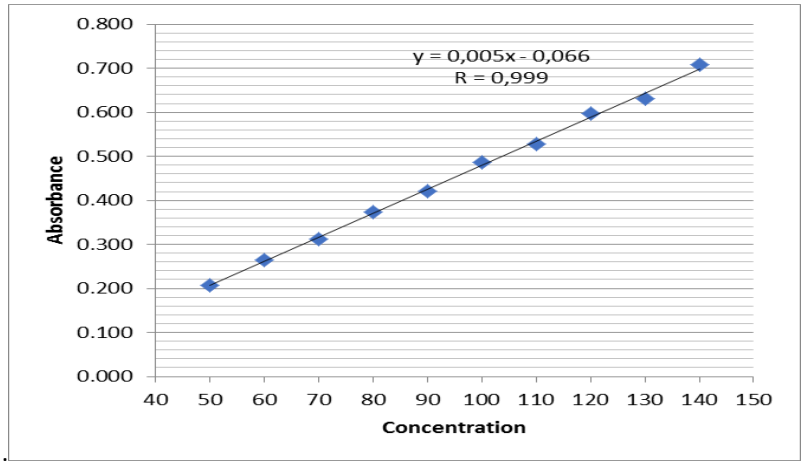

Figure 2. Standard curve of Quercetin

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The regression equation obtained from standard curve of quercetin was Y=0.005x + (-0.066) with coefficient correlation value (r) of 0.999. This value of coefficient correlation state that there was a 99.9% of correlation between the concentration of quercetin and its absorbance. Widyastuti (2010) also gave the same result in determination of total flavonoid using quercetin standard curve. RSD value of this study was 2-7%, meet the requirement of RSD value as in AOAC (Gonzales *et al,* 2010). Therefore it can be concluded that validation requirement especially precision was fulfilled. Furthermore the regression equation was used for the determination of total flavonoid in Kasturi extract with 3 different concentrations. The result obtained is shown in Table 1.

Tabel 1. Result from the determination of total flavonoid content in ethanolic extract of Kasturi

Kasturi extract concentration (%)	The mean levels of total flavonoid (% b/b)
30	1.64
50	2.60
70	3.50

FTIR spectra of ethanolic extract of Kasturi

FTIR spectra profile of certain extract of medicinal herb was fingerprint pattern obtained from the total constituents with a variety of secondary metabolites (Nauman, 1998). Fig. 3 shows FTIR spectra of ethanolic extract of Kasturi obtained from FTIR ABB Horizon MB 2000. Based on FTIR spectra, it was shown that each extract have an almost identical FTIR spectra profile at wavenumbers of 3300 cm^{-1} and 1710 cm^{-1}, coming from stretching vibration of –OH group and the C=O carbonyl, recpectively which are the main group of flavonoid (Molyneux, 2010). The difference of absorbption peaks was affected by the concentration of each flavonoid. The main differences of Absorption also shown at the fingerprint region. However, the subttle and small difference of the absorbance and the characteristic of constituent absorption were easier to be investigated using chemometrics approach such as principal component analysis (Rohman *et al*, 2010).

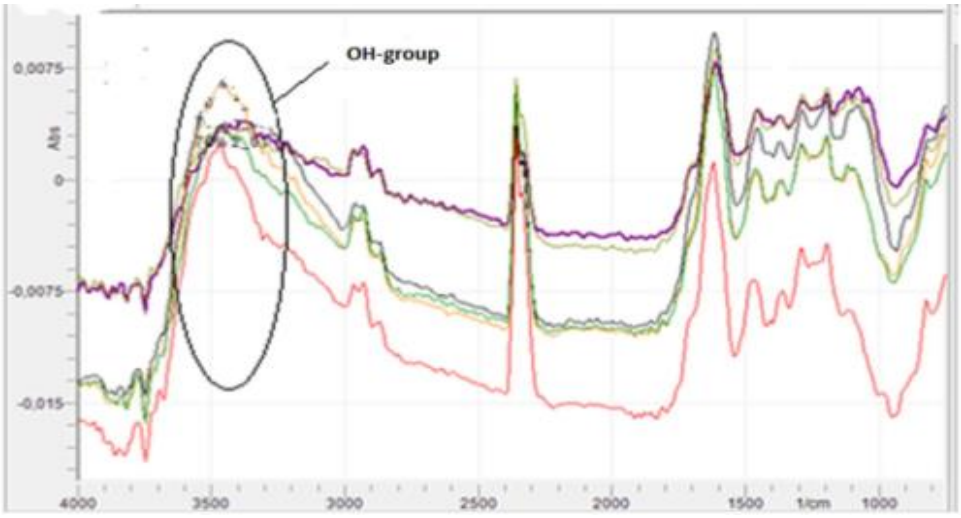

Figure 3. FTIR Spectrum of ethanolic extract of Kasturi

Determination of Total Flavonoid content of ethanolic extract of Kasturi

Determination of total flavonoid content using FTIR spectroscopy is aided by multivariate calibration of PLS. PLS will give automaticaly the spesific and relevant information to the chemical characteristic from the calibration model at certain wavenumber (Van de Vort *et al*, 1992). Therefore, the selection of wavenumber was based on optimation process which give the linear correlation of the absorption shifting at some certain wavenumber (Triyasmono *et al*, 2013). The determination of total flavonoid content of Kasturi extract in 3 different concentrations were carried out at wavenumber of 3300 cm^{-1}. This peak originates from –OH functional group of flavonoid (Rohaeti dkk., 2011; Molyneux, 2010; Desmiaty, 2009). The x-axis was total

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flavonoid concentration of ethanolic extract obtained from $AlCl_3$ method, whereas the y-axis was the absorbance of Kasturi's FTIR spectra at wavenumber of 3300 – 3700 cm^{-1}. The PLS calibration model shows the coefficient determination (R^2) value was 0.998 and RMSEC value of 0.20%. The high value of R2 and low value of RMSEC indicated that the calibration model was acceptable (Miller and Miller, 2010; Baranska et al., 2005). The regression plot for the correlation between actual value of flavonoid content and FTIR predicted value can be seen on Fig. 4.

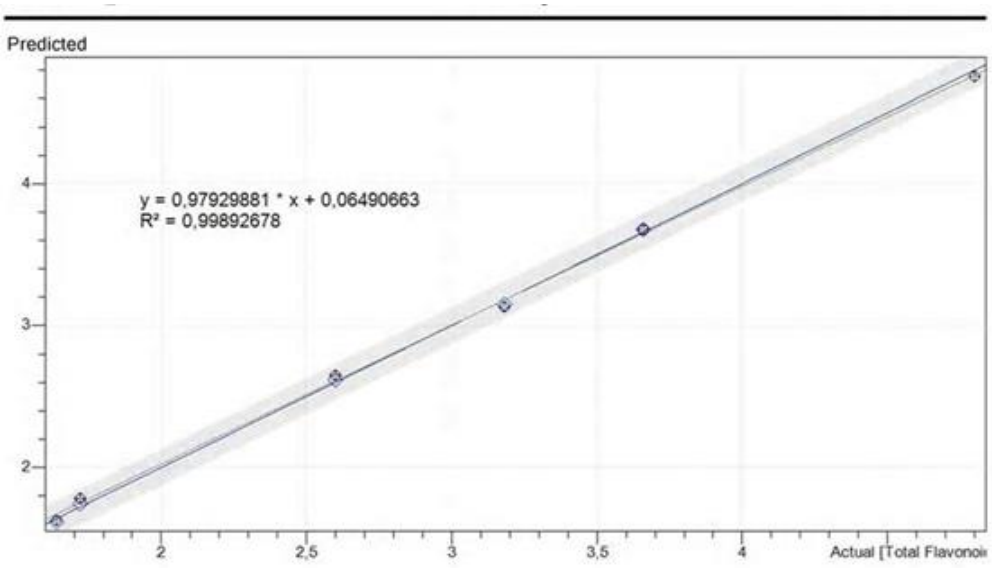

Figure 4. PLS regression plot for the relationship between of actual value (x-axis) and FTIR predicted value (y-axis) of flavonoid content in ethanolic extract of Kasturi in calibration model.

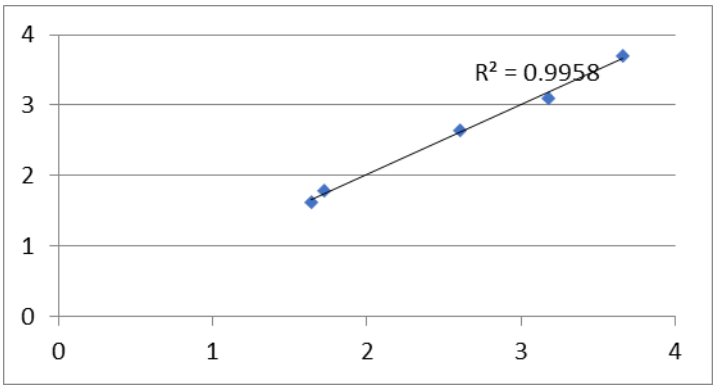

Figure 5. PLS regression plot for the relationship between of actual value (x-axis) and FTIR predicted value (y-axis) of flavonoid content in ethanolic extract of Kasturi in validation model.

The validity of the model was evaluated using cross validation technique. This technique was useful to determine the optimum number of component from a small independent sample (Stchur *et al*, 2012). Based on the cross validation result for determining accuracy and prediction of the calibration model showed R^2 value was 0.995 and RMSEP value of 0.831, as shown in Fig. 5. The plot of validation model shows good model which produce adjacent points along the regression line with a slope value about 1 (Naes *et al*, 2002). The high R^2 value and low RMSEP value show that the accuracy and precision of the developed model is accepted (Miller and Miller, 2010). Based on this result, it can be stated that FTIR spectroscopy combined with PLS regression can be used as an alternative method for rapid analysis of total flavonoid content.

CONCLUSION

FTIR spectroscopy combined with PLS regression can be used as an alternative method for the determination of total flavonoid content. The calibration and validation model resulted the coefficient

determination (R^2) of 0.998 and 0.995, respectively. The RMSEC and RMSEP values of the developed model were 0.205 and 0.831, respectively. Based on the findings, it can be concluded that FTIR spectrum of ethanolic extract of Kasturi can be used accurately and precisely to predict the total flavonoid content of kasturi with the regression equation of predicted value (y) = 0.979 x actual value as determined by $AlCl_3$ method + 0.065.

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