Global Journal of Medicinal Plants Research

ISSN: xxxx-xxxx Vol. 1 (1),
pp. 067-074, December, 2013. ©
Global Science Research Journals

Full Length Research Paper

Determination of catechin as main bioactive component of gambir (*Uncaria gambir* Roxb) by FT-NIR Spectroscopy

Andasuryani[1,2], Y. A. Purwanto[3], I. W. Budiastra[3] and K. Syamsu[4]

[1]Department of Agricultural Engineering, Faculty of Agricultural Technology, Andalas University, Padang, Indonesia. [2]Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia.
[3]Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia.
[4]Department of Agro industrial Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia.

Accepted 19 October, 2013

Catechin, an abundant component within gambir, is the main determinant of gambir quality. The general method to determine catechin is time consuming, costly and produces chemical residual. The objective of this study was to develop NIR calibration model which could be used to predict catechin content of gambir efficiently. Calibration model was established using partial least square (PLS) algorithm by investigating 3 different pre-treatment methods which were normalization between 0 and 1 (n01) first derivative Savitzky-Golay 9 points (dg1) and n01 in combination with dg1 (n01, dg1). Determination of optimum factors number was conducted based on predicted residual error sum square in validation set (V-set PRESS) values and consistency. This study found that pre-treatment n01, dg1 was the best method to produce calibration model. Quality of the best model was shown by (1) high coefficient correlation (r) = 0.91, (2) low standard error of calibration set (SEC) = 2.53% and standard error of validation set (SEP) = 2.44%, (3) slight difference between SEC and SEP. This study demonstrated that FT-NIR spectroscopy could be used as a tool for predicting the catechin content in gambir. It could replace expensiveness and time as well as effort consuming reference method.

Key words: Fourier transform-near infrared (FT-NIR) spectroscopy, gambir, catechin, partial least squares (PLS).

INTRODUCTION

Uncaria gambir (Roxb.), a native plant of Indonesia and Malaysia is a member of family Rubiaceace and genus *Uncaria* (Heitzman et al., 2005). Leaves and young twigs of *U. gambir* can be extracted to produce gambir. Averagely, 76% of gambir is catechin (Taniguchi et al., 2007a), a polifenol compound with flavonoid structure. As consequence of its potential as anti oxidant and anti bacteria, catechin is most likely used as a raw material of pharmaceutical and cosmetic product. In Southeast Asia, gambir is often used as remedies for diarrhoea and sore throat (Taniguchi et al., 2007b). Apea-Bah et al. (2009) also reported that gambir is used for diarrhoea and dysentery treatment traditionally.

General methods for determining catechin content quantitatively are ultraviolet spectrophotometric and high performance liquid chromatography (HPLC). However,

*Corresponding author. E-mail: mshtaiwi@kau.edu.sa and asuryani@yahoo.com

these methods are inefficient since they need more time, high cost and produce chemical waste. Near infrared (NIR) spectroscopy has decisive advantages compared to traditional methods, whereby it can analyse sample rapidly (a few seconds per sample), it does not need sample preparation, it is chemical-free (limited to the reagents required for reference analyses), has no resultant waste and can be carried out on-line (Pissard et al., 2012; Saleh, 2012). Some research has found the capability of this technology to determine bioactive compound, such as the determination of alkaloids and phenolic substances in green tea leaves (Schultz et al*.,* 1999), procyanidins in cocoa (Whitacre et al., 2003), condensed tannins and dry matter in red grape homogenates (Cozzolino et al., 2008), main catehins contents in green tea (Chen et al., 2009), flavanols in grape seeds (Gallego et al., 2010), and phenolic compounds of grape skins during ripening (Gallego et al., 2011). Though it is widely used to determine bioactive compound of material; NIR spectroscopy has not been applied to measure catechin content in gambir. As consequence of complexity of NIR Spectra, information can not be obtained easily for determining single component of analysed material. One of the popular standard methods to develop NIR calibration model is partial least square (PLS) which was used in this study. The objective of this study was to develop NIR calibration model which can be used to predict catechin content with PLS algorithm.

MATERIALS AND METHODS

Sample preparation

This study used 162 samples, obtained from Siguntur, an area in Pesisir Selatan district, West Sumatera province, Indonesia. Initially, the samples form was solid. It was crushed using a mortar and sieved through number 30-sieve (600 µm) screen (Feng et al., 2008). Powder resulted from this process was used as material for analysis.

Chemical analysis

Chemical analysis was conducted to measure catechin content in gambir. Data obtained from chemical analysis were used as reference to develop model calibration. To analyse reference catechin content, this study utilized statistical parameters such as average, maximum, minimum, deviation standard, and skewness ratio analysis. Standard deviation is the standard error of reference data. Skewness ratio analysis was conducted to test normality of data distribution of catechin content.

Determining catechin content in gambir is conducted by referring to Indonesia National Standard (SNI 01-3391-2000) (Hayani, 2003). Catechin as one of the main components in gambir, is soluble in ethyl acetate completely. Absorption of gambir solution in ethyl acetate at a wavelength of 279 nm is proportional with catechin content in gambir. Measurement was conducted by comparing absorption of gambir sample solution with catechin standard solution using Spectrophotometer U-2010, Hitachi.

Measurement of catechin content is conducted through several stages (Hayani, 2003).

Preparation of catechin standard

Before measurement, catechin standard is dried in an oven at temperature 105°C for 3 h. Fifty milligrams dry catechin is filled into a 50 ml flask. It is dissolved and diluted with ethyl acetate until reach the calibration mark (solution A). Put the solution A into ultrasonic bath for 5 min to make it homogeneous. Put 2 ml of solution A into a 100 ml Erlenmeyer with lid and add 50 ml of ethyl acetate (solution B). After putting the solution B into ultrasonic bath for 5 min, the solution can be used for measurement;

Preparation of gambir sample

The sample is crushed with mortar as soft as possible. Create a gambir layer as thin as possible above petri dish and dry it into an oven at temperature 105°C for 3 h up to 15 to 17% weight loss. Put 50 mg sample of dry gambir into 50 ml flasks. It is dissolved and diluted with ethyl acetate until it reached the calibration mark (solution C). Put the solution C into ultrasonic bath for 5 min. Then the solution is filtered. Throw 15 ml filtrate from this first filter and continue filtering. Put 2 ml of filtrate from solution C into 100 ml Erlenmeyer lid and add 50 ml of ethyl acetate solvent (solution D). Put solution D in the ultrasonic bath for 5 min before measurement;

Measurement of solution

Measurement is conducted using ultraviolet spectrophotometer at 270 and 300 nm wavelength. It is started from solution ethyl acetate equals with zero. Then, measure the absorbance of the standard solution and the absorbance of the sample solution. Catechin content was counted by the formula:

$$\text{Catechin (\%)} = \frac{\text{Et }279}{\text{Ec }279} \times \frac{\text{Ws}}{\text{W}} \times 100$$

whereby Et 279 is absorbance of gambir sample solution at 279 nm wavelength, Ec 279 is absorbance of catechin standard solution at 279 nm wavelength, Ws is catechin standard weight (g) and W is weight of gambir sample (g). Catechin standard (≥ 98%) were purchased from Sigma Co., USA.

NIR spectroscopy analysis and chemometrics

NIR spectroscopy analysis was carried out using Buchi NIRFlex N-500 solids with resolution of 4 cm^{-1} and 8 scans per sample. Each sample was placed in a petri dish and scanned by three roundtrips petri dish in a different position. Average spectra were used for calibration. Spectra data were collected by measuring the diffuse reflectance of sample whereby each spectrum is measured every 4 cm^{-1} data intervals in range from 10000 to 4000 cm^{-1}, thus there were 1501 variable spectrum. Operation of the instrument and data collection of NIR spectra were conducted by using NIRWare 1.2 software (Büchi Labortechnik AG, Flawil, Switzerland) with room temperature of 25°C.

Based on reference value from laboratory, all the 162 samples were divided into two groups randomly. The first group was named set calibration (C-Set) consisting 2/3 of samples which was 108 samples. This group was used for model development. Meanwhile, the other 1/3 was named set validation (V-Set) with 54 samples. This group was used for model testing.

Chemometric analysis was conducted using NIRCal 5.2 software (Büchi Labortechnik AG, Flawil, Switzerland). Calibration model was established using PLS algorithm which used one to fifteen PLS factors or latent variables to develop calibration model. Determining optimal number of PLS factors were conducted based on PRESS within validation set (V-Set PRESS) value and consistency. The number of PLS factors selected should obtain minimum V-Set PRESS and consistency between 80 and 110% (NIRCal 5.2 manual, 2007).

Determining of pre-treatment spectra to develop NIR calibration model depends on material type and content to be predicted (Mouazen et al., 2005). Pre-treatment normalization between 0 and 1 (n01) can reduce baseline variation (NIRCal 5.2 manual, 2007). Meanwhile, first derivative Savitzky-Golay 9 points (dg1) can be more effective for very sharp absorption band spectra with high noise to detect shifting of very small wavelength (NIRCal 5.2 manual, 2007). Udelhoven et al. (2003) acknowledge that spectra variation caused by different grain size distributions can be reduced by normalisation of spectra. The peaks and valleys become clear by dg1 compare to original spectra which is not very obvious (Cen and He, 2007). To obtain the best calibration model, this study applied three different pre-treatment spectra which were (1) n01, (2) dg1, and (3) n01 in combination with dg1 (n01, dg1). In addition, the resulted models were compared with original model.

Statistical parameters used to evaluate the developed NIR calibration model were:

a) Bias, that is, the average deviation between the reference value (x_n) and the predicted value (y_n) of V-Set. Williams and Norris (1990) recommend that bias should be equal to zero.

$$\mathrm{Bias} = \frac{1}{N}\sum(x_n - y_n)$$

b) The standard error of calibration set (SEC), that is, the standard deviation of the differences between the reference value (x_n) and the predicted value (y_n) of C-Set.

$$\mathrm{SEC} = \sqrt{\frac{1}{N-1}\sum(x_n - y_n)^2}$$

c) The standard error of validation set (SEP), that is, the standard deviation of the differences between the reference value (x_n) and the predicted value (y_n) of V-Set, corrected for bias.

$$\mathrm{SEP} = \sqrt{\frac{1}{N-1}\sum(x_n - y_n - \mathrm{Bias})^2}$$

d) Predicted residual error sum square (PRESS), that is, sum square of the deviation between the reference value (x_n) and the predicted value (y_n).

$$\mathrm{PRESS} = \sum(x_n - y_n)^2$$

e) Coefficient of correlation (r) between the reference value (x_n) and the predicted value (y_n). The model is considered more useful when r value approaches 1, whereby r value is larger than 0.90, considered as high correlation (Williams and Norris, 1990).

$$r = \frac{\sum(x_n - \bar{x}_n)(y_n - \bar{y}_n)}{\sqrt{\sum(x_n - \bar{x}_n)^2 \sum(y_n - \bar{y}_n)^2}}$$

f) Coefficient of determination (R^2) between the reference value (x_n) and the predicted value (y_n).

$$R^2 = \left(\frac{\sum(x_n - \bar{x}_n)(y_n - \bar{y}_n)}{\sqrt{\sum(x_n - \bar{x}_n)^2 \sum(y_n - \bar{y}_n)^2}}\right)^2$$

g) Coefficient of variation (CV): A very reliable calibration can be achieved when the value of CV in C-Set is lower than 5% and the value of CV in V-Set is lower than 10% (Mlček et al., 2006).

CV in C-Set: $\mathrm{CV} = \frac{\mathrm{SEC}}{\bar{x}} \text{ x } 100$

CV in V-Set: $\mathrm{CV} = \frac{\mathrm{SEP}}{\bar{x}} \text{ x } 100$

h) Ratio of Performance to Deviation (RPD), that is, express as the ratio of standard deviation of the reference analysis of the investigation to the standard error of the estimate. RPD values below 1.5 indicates that the calibration cannot be used, between 1.5 and 2.0 is possible to distinguish high and low values, between 2.0 and 2.5 is possible for quantitative prediction, while values between 2.5 and 3.0 and above 3.0 is considered good and very good, respectively (Mouzan et al., 2005).

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{SEP}}$$

h) Consistency, that is, the relationship between SEC and SEP. A value of 100% is optimal (standard error of the C-Set and V-Set is equal) and between 80 and 110% is still acceptable (NIRCal 5.2 manual, 2007).

$$\mathrm{Consistency} = \frac{\mathrm{SEC}}{\mathrm{SEP}} \text{ x } 100$$

RESULTS

Chemical data

Reference values of catechin content in gambir at the C-Set and V-Set are shown in Table 1. Catechin content values of V-set were ranging in value of C-set. These values were fit for NIR calibration, because developing a model requires data set of calibration which covers a range of concentration. In addition, both set calibration and set validation obtained skewness ratio between -2 and 2 which indicated that catachin data were distributed normally. Differentiation of deviation standard between C-set and V-set was not significant. Therefore, data distribution of C-set and V-set could be considered similar. Calibration model would be valid to predict concentration if the test was done for similar sample.

Spectra evaluation

Figure 1 shows the original spectra of gambir. The original one shows that there is a parallel shift of spectra. It occurred because spectra data of NIR did not only

Table 1. Reference values of catechin content in gambir (% w/w).

	N	Minimum	Maximum	Mean	Skewness ratio	SD
Calibration set	108	48.81	77.02	61.62	0.79	6.15
Validation set	54	50.01	75.95	61.35	1.65	5.71

N: sample number; SD: standard deviation.

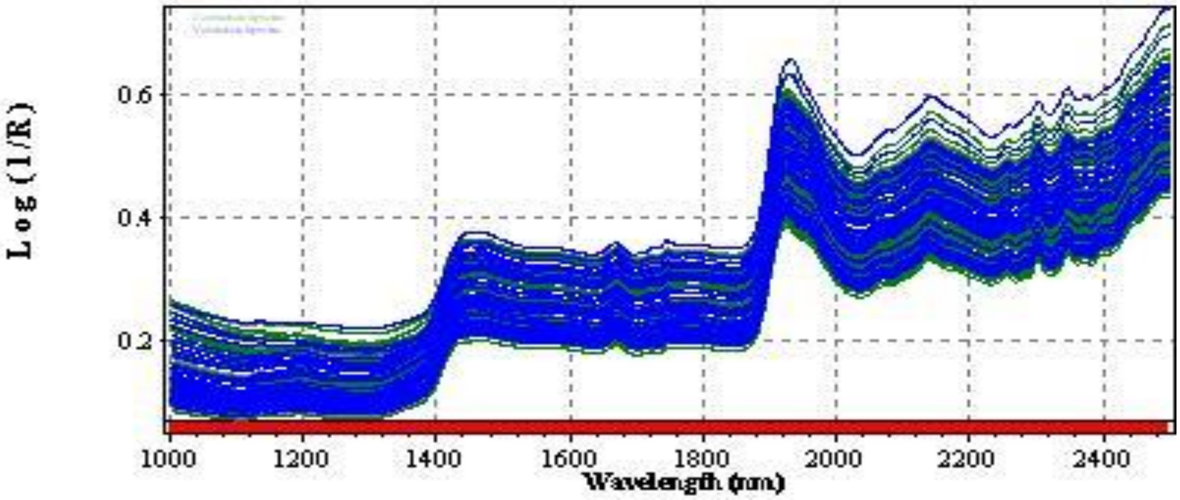

Figure 1. The original spectra of gambir.

Table 2. Calibration and validations statistics of NIR model for catechin content in gambir.

Pre-treatment	Calibration set				Validation set				
	F	SEC (%)	R	CV (%)	SEP (%)	R	CV (%)	Bias (%)	RPD
n01	8	3.66	0.80	5.95	3.59	0.79	5.85	0.63	1.61
dg1	7	2.64	0.90	4.29	2.54	0.90	4.13	0.15	2.28
n01, dg1	7	2.53	0.91	4.12	2.44	0.91	3.97	0.19	2.37
Original	12	2.90	0.88	4.72	3.00	0.87	4.89	0.96	1.92

F: PLS factor; SEC: standard error in calibration set; SEP: standard error in validation set; r: coefficient of correlation; CV: coefficient of variation; RPD: ratio of performance to deviation.

contain sample information, but also background information as well as noises. Therefore, pre-treatment spectra were needed before modelling to get reliable and accurate model. Spectra data that resulted from diffuse reflectance measurement at solid sample would be followed by scattering noise as a result of particle size difference (Chen et al., 2013).

Calibration development

Choosing the best model is difficult, as it depends on a number of parameters: SEC and SEP is the difference in explained y-variance between the calibration and the validation set, the difference between SEC and SEC is the correlation coefficient between the predicted and the measured values, the number of latent variables, etc (Lammertyn et al., 2000). To obtain the best model for predicting catechin content, several pre-treatments to NIR spectra. Effect of the treatments is shown in Table 2.

Selecting number of PLS factors and pre-treatment spectra are an important step while developing calibration model with PLS algorithm. The optimal number of PLS factors will be selected if the V-set PRESS value was minimal and consistency value between 80 and 110%. This study demonstrated that the optimal number of factor obtained while using n01 pre-treatment was 8. This number provided V-set PRESS value 706.13 and consistency 101.95%. By using dg1 pre-treatment, the model obtained 7 optimal factors. This number provided V-set PRESS value and consistency of 342.43 and 104.08%, respectively. While using n01 in combination

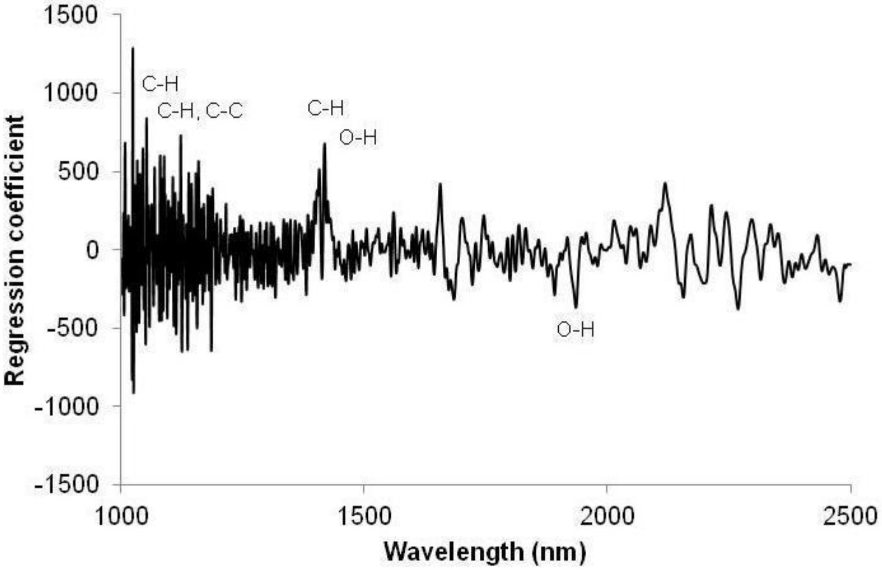

Figure 2. Regression coefficient used to develop the calibration for catechin in gambir.

with dg1 pre-treatment, the model obtained 7 optimal factor numbers which provided V-set PRESS value 317.52 and consistency 103.83%. Meanwhile, optimal factor number obtained while using original for NIR spectra analysis was 12, which generate V-set PRESS value and consistency of 528.76 and 96.67%, respectively. It seems that the original model needed larger number of factor than model using pre-treatment spectra. As consequence of the existence noise in the original model, the noise was also modelled.

SEP and SEC were precise parameters of NIR calibration model. All the pre-processing spectra produced lower values of SEP and SEC compare to standard deviation of reference data (Table 1). SEC and SEP values resulted by using n01 in combination with dg1 pre-treatment were smaller than n01 pre-treatment, dg1 pre-treatment and the original. A good model should have a low SEC, a low SEP, a high correlation coefficient, but also a small difference between SEC and SEP (Jha, 2010). A large difference is an indication that calibration set is not a representative of validation set (Lammertyn et al., 2000). Therefore, the resulted model using n01 in combination with dg1 pre-treatment was more precise than n01 pre-treatment, dg1 pre-treatment and the original.

Bias is a parameter which explains accuracy of calibration model. It shows differences between value of NIR prediction and actual value. The resulted model using dg1 pre-treatment and n01 in combination with dg1 pre-treatment was more accurate than n01 pre-treatment and original as its bias value was close to zero (Williams and Norris, 1990). Value of r and CV are parameters of calibration model fitness. This study found that only the model using n01 in combination with dg1 pre-treatment which had r value larger than 0.90. Model with r > 0.90 reveals a good correlation between NIR spectra and actual catechin content (Williams and Norris, 1990). A model with CV in C-Set is lower than 5% and the value of CV in V-Set is lower than 10% and reveals a very reliable calibration (Mlček et al., 2006). As consequences of CV is the lowest value of model using n01 in combination with dg1 pre-treatment among the models, it could predict catechin content in a new gambir data set properly. CV value mostly depends on source of data.

RPD is the ratio between standard deviation reference and SEP. It indicates capability of calibration model to predict desired components. Its value depends on variability of reference data. The greater the variability, the larger the RPD value obtained. The best RPD value resulted from model using n01 in combination with dg1 pre-treatment, which was 2.37. RPD value from 2.00 to 2.50 indicates that quantitative prediction could be possible to new samples (Mouazen et al., 2005). Though RPD value was not high, this study obtained SEP value (2.44%) was lower than standard deviation of reference data (5.71%).

Based on model parameters performances (Table 2), the best model obtained was the model using n01 in combination with dg1 pre-treatment spectra as it could eliminate spectra variability that resulted from differences of particle size. In addition, it could remove other components unless catechin and rise components which correlate with catechin content. Figure 2 describes regression coefficient of the optimal PLS model to predict catechin content in gambir (PLS factor = 7). The highest regression coefficient was around 1052 nm (C-H Str.plus def., CH_2), at 1080 nm (C-H Str.plus C-C Str., benzene), at 1143 nm (C-H Str. second overtone, aromatic), at 1416 nm (C-H Str.plus def., CH_2), at 1418 nm (C-H Str.plus def., aromatic), and at 1420 nm (O-H Str. First overtone,

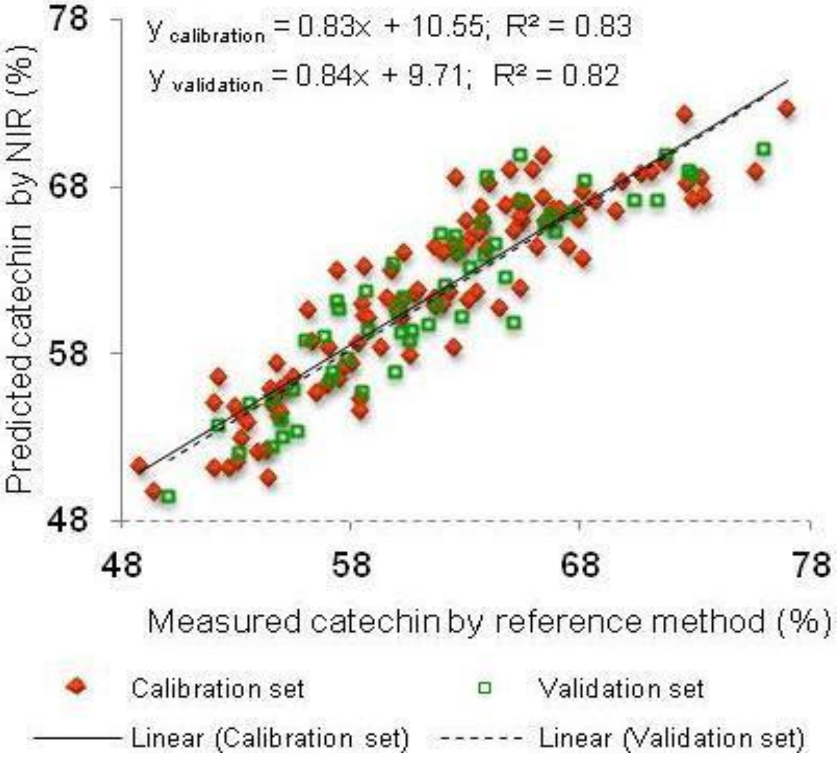

Figure 3. Reference value versus NIR predicted for catechin content.

ArOH), and at 1940 nm (O-H Str. Plus def., H_2O) (Osborne et al., 1993). These values were influenced by hydric groups (such as C–H and O–H) which could be found most in molecule structures of catechin. Figure 3 reveals relationship between catechin actual value and NIR prediction value with equation $y_{calibration}$ = 0.83x + 10.55 with determination coefficient (R^2) 0.83 and $y_{validation}$ = 0.84x + 9.71 with determination coefficient (R^2) 0.82. R^2 between 0.82 and 0.90 was considered to be a good prediction (Mouazen et al., 2005).

Some researchers acknowledge that pre-treatment method conducted before modelling will increase calibration result (Schulz et al., 1999; Udelhoven et al., 2002). This study also found that dg1 pre-treatment and n01 in combination with dg1 pre-treatment spectra could increase calibration model. However, n01 pre-treatment spectra could not increase the calibration model. This finding supported by Buddenbaum et al. (2012) who acknowledges that PLSR is a powerful regression tool which employs full spectra range, can find essential information of spectra, and does not depend on spectral pre-treatment which emphasize on information contained in the spectra.

DISCUSSION

Feature of NIR spectra of gambir (Figure 1) looked similar with spectra of green tea leaves as demonstrated by Chen et al. (2009). The similarity was caused by both gambir and green tea leaves contained catechin. The main catechin compounds in green tea leaves are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Meanwhile, gambir has (±) catechin and (+) epicatechin. Chen et al. (2009) find that some peaks of spectra occur at region 4000 to 8500 cm^{-1} (2500 to 1176 nm), meanwhile, this study found it at region 4000 to 8333 cm^{-1} (2500 to 1200 nm). Though there were similarities of spectra feature, NIR spectra were not easy to interpret. To solve this problem, every type of commodity needed a new calibration model to obtain a good result.

This study only analyzes (±) catechin which is abundant in gambir. However, Chen et al. (2009) analyze some types of catechin in green tea leaves and Chen et al. (2010) analyze in tea polyphenols (TPs) powder extracted from the green tea. The best model resulted from this study had correlation coefficient above 0.90 which was 0.91. Chen et al. (2009) also obtain correlation coefficient above 0.90 which is from 0.9763 to 0.9859 for EGC, EC, EGCG and ECG. Meanwhile, Chen et al. (2010) obtained correlation coefficient for catechin gallate (CG), (-)-epicatechin gallate (ECG), and (-) epigallocatechin gallate (EGCG) above 0.9 whichis from 0.934 to 0.996 and (+)-catechin (D,L-C), (-)-epicatechin (EC) and (-)-epigallocatechin (EGC) below 0.9 which is frm 0.69 to 0.80. The low values of Chen's finding occur due to low contents of catechins and their instability. This is the main reasons why their models are poor.

Standard error of this study is larger than Chen et al. (2009) study. However, the standard error was alike with Chen et al. (2010) for EGCG component only. Nevertheless, this study obtained 7 PLS factors with minimal V-set PRESS and consistency value range in from 80 to 110%. Consistency value in this range indicates that the number of PLS factor is optimum (Elfadl et al., 2010). It could prevent underfitting as well as overfitting. Meanwhile, Chen et al. (2009) and Chen et al. (2010) does not discuss consistency value.

Conclusions

This study found that catechin content in gambir could be determined by FT-NIR spectroscopy along with chemometric tools. The optimum number of PLS factors and pre-treatment spectra were determined based on V-set PRESS and consistency values. The best final PLS model resulted by n01 in combination with dg1 pretreatment spectra and 7 PLS factors. FT-NIR spectroscopy is an easy and rapid technique and can replace conventional method compared to the conventional method.

ACKNOWLEDGEMENT

The authors express their appreciation for the financial support by Strategic Research Grant of DGHE Ministry of Education and Culture, Republic of Indonesia No. 42/I3.24.4/SPK-PUS/IPB/2012 (01 March 2012).

ABBREVIATIONS

NIR, Near infrared; **PLS,** partial least squares; **r,** correlation coefficient; **SEC,** standard error of calibration set; **SEP,** standard error of validation set; **CV,** coefficient variant; **V-Set-PRESS,** predicted residual error sum square of validation set; **RPD,** ratio of performance to deviation; **n01,** normalization between 0 and 1; **dg1,** first derivative Savitzky-Golay 9 points.

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