

## FTIR and Chemometrics Application on Determination of Total Flavonoid Content of Pasak Bumi Root Extract (*Eurycoma longifolia* Jack.)

Liling Triyasmono<sup>1\*</sup>, Ana Ulfah<sup>1</sup>, M. Ikhwan Rizki<sup>1</sup>, Khoerul Anwar<sup>1</sup>,  
Totok Wianto<sup>2</sup>, Heri Budi Santoso<sup>3</sup>

<sup>1</sup>Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru, Indonesia.

<sup>2</sup>Departement of Physic, Faculty of Mathematics and Natural Sciences Universitas Lambung Mangkurat, Banjarbaru, Indonesia.

<sup>3</sup>Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru, Indonesia.

\*Email: [liling.triyasmono@ulm.ac.id](mailto:liling.triyasmono@ulm.ac.id)

### ABSTRACT

The combination of FTIR and chemometrics is an alternative method on determination of total flavonoid content of pasak bumi (*Eurycoma longifolia* Jack.) root extract. This study aims to determine the method of FTIR and chemometrics can be used for determination of total flavonoid content and determine the total flavonoid content of *E. longifolia* root extract using FTIR and chemometrics. The samples from three growing area were determined their flavonoid content by colorimetric method and measured their absorbance by FTIR spectrophotometer. The analysis was done by PCA chemometrics to grouping IR spectra based on growing area and PLSR to determine prediction model of total flavonoid content of *E. longifolia* root extract. The best grouping and prediction model is shown by IR spectra in the range of wavenumbers 1800-1540 cm<sup>-1</sup> with total variation is 99% and prediction model with equation  $y = 0.995x + 0.002$  (R<sup>2</sup> calibration = 0.995; R<sup>2</sup> validation = 0.970; RMSEC = 0.008; RMSECV = 0.021). The total flavonoid content of *E. longifolia* root extract (% b/b ± SD) from Mandiangin, Condong, and Sabuai is  $0.225 \pm 0.009$ ;  $0.437 \pm 0.007$ ; and  $0.466 \pm 0.016$  (R<sup>2</sup> = 0.995 and RMSEP = 0.008). Based on this, the combination of FTIR and chemometrics can be used to predict the total flavonoid content of unknown *E. longifolia* root extract

**Keywords:** *Eurycoma longifolia* Jack., Flavonoid, FTIR, PCA, PLSR

## I. INTRODUCTION

Pasak bumi (*Eurycoma longifolia* Jack.) is a medicinal plant that can be found in tropical forests of Asian countries. Pasak bumi in Indonesia are widely found in the Kalimantan and Sumatra forests. Pasak bumi root is used by the community as an aphrodisiac, antipyretic, antimalarial, and treats dysentery (Hadad & Taryono, 1998). The compounds contained in pasak bumi root are eurikomanon, kuasinoid, flavonoids, phenolics and terpenoids (Khanam *et al.*, 2015). Flavonoid content in the pasak bumi has antioxidant activity, inhibits the activity of the tyrosinase enzyme and is able to inhibit nuclear factor kappa B (NF- $\kappa$ B) which binds to DNA as an anti-inflammatory action in cancer (Hassan *et al.*, 2015; Tran *et al.*, 2014; Varghese *et al.*, 2013).

The standard method used in determining the total flavonoid content in medicinal plant extracts is the colorimetric method using UV-Vis spectrophotometer (Depkes RI, 2000). However, this method requires reagent and a lot of time. Fourier Transformed Infrared (FTIR) is an alternative in determining the total flavonoid content of a plant because it is able to measure several samples at once, measurement is fast, and non-destructive (Hermanto *et al.*, 2015). The FTIR spectra produced is very complex so the use of chemometrics will facilitate analysis. Chemometrics is a multivariate statistical

method that is useful for processing information obtained from infrared spectra into information that is useful for qualitative and quantitative analysis.

Several studies that have successfully used a combination of FTIR and chemometrics are the determination of tempuyung and bungur total flavonoid contents (Rohaeti *et al.*, 2011; Triyasmono *et al.*, 2015), identification and authentication of red ginger (Purwakusumah *et al.*, 2014) and purity detection of Zamzam water (Rasyida *et al.*, 2014). This study will use a combination of FTIR and chemometrics consisting of PCA for grouping IR spectra and PLSR to determine the total flavonoid content of pasak bumi root by connecting the total flavonoid content using the standard method (UV-Vis spectrophotometry) with the absorbance of pasak bumi root extract using FTIR. The parameters of acceptability by the total value of variation, correlation and error obtained.

This study aims to determine the method of FTIR and chemometrics can be used for determination of total flavonoid content and determine the total flavonoid content of *E. longifolia* root extract using FTIR and chemometrics

## II. METHOD

### A. Materials

Pasak bumi root were collected from three areas with different growing heights, namely Mandiangin, South

Kalimantan (1200 meters above sea levels (masl)); Condong, West Kalimantan (20 masl); and Sabuai, Central Kalimantan (2 masl). Aerosil, AlCl<sub>3</sub> 10% (Merck), ammonia, acetic acid 5%, concentrated glacial acetic acid (Merck), aquadest, ethanol 70%, ethanol p.a (Merck), hydrochloride acid, quercetin (Sigma-Aldrich), methanol p.a (Merck), sodium hydroxide, lead acetate 10% dan potassium bromide for IR spectroscopy.

FTIR-ATR Spectrophotometer (Alpha) and UV-Vis Spectrophotometer (Spectronic Genesys 10uv) was used for measuring the analytical response.

## **B. Preparation of Simplicia Powder**

The cleaned root were cut into small pieces to speed up the drying process. The sample was dried using an oven at a temperature of 60°C until constant weight was obtained. dried samples were powdered and passed through sieve 25.

## **C. Preparation of Pasak Bumi Root Extract**

The simplicia powder (150 grams) were extracted by maceration using 70% ethanol solvent for 3x24 hours at room temperature. The solvent replaced every 1x24 hours of sample immersion. The ratio of samples and solvents used was 1:10 for 2x24 hours and 1:5 in the last 24 hours. The sample was filtered using filter paper. The filtrate was evaporated with a vacuum

rotary evaporator at a temperature of 60°C and then concentrated until a constant weight is obtained. Viscous extract stored in a closed container and the % yield was calculated

## **D. Drying of Pasak Bumi Root Extract**

Viscous extract of pasak bumi root was mixed with aerosil as an adsorbent. Viscous extract and aerosil were crushed to form a dry extract. Work was carried out in a humidity-controlled room ( $\pm$  40%). The dried extract was then drying at 50°C for 1 hour to remove the remaining solvents. Extracts were put into a vial and stored in a desiccator

## **E. Identification of Flavonoids**

Identification of flavonoids was carried out on viscous extract of pasak bumi root using alkali reagent, lead acetate 10% and dripping extract on filter paper and evaporated with ammonia.

## **F. Determination of Total Flavonoid content with UV-Vis Spectrophotometer**

The viscous extract of the pasak bumi root were dissolved with ethanol p.a (10000 ppm for the sample of Condong and Sabuai and 30000 ppm for the Mandiingin sample). Each extract solution was taken as much as 0.5 mL and reacted with 1.5 mL ethanol p.a; 0.1 mL 10% AlCl<sub>3</sub> (in concentrated glacial acetic acid); 0.1 mL of 5% acetic acid (in methanol p.a) and 2.8

mL of aquadest. The solution was allowed to stand for 20 minutes then the absorbance was measured at the maximum wavelength of 418 nm

### **G. Determination of Total Flavonoid content for FTIR-ATR Spectrophotometers**

The dried extracts (50.0 mg) were mixed with 950.0 mg of KBr powders until homogeneous. The samples were then measured using FTIR-ATR with scanning 32 times and a resolution of  $2\text{ cm}^{-1}$  in the middle IR area ( $4000\text{-}400\text{ cm}^{-1}$ ). Spectra are recorded as absorbance values (Triyasmono *et al.*, 2017).

### **H. Analysis using Chemometrics**

Spectra data in the form of absorbance was processed using the software The Unscrambler X 10.4 (Camo Inc.). The analysis consisted of qualitative analysis using the PCA (Principal Component Analysis) technique to grouping IR spectra of samples based on the place of growth and quantitative analysis using PLSR (Partial Least Square Regression) to determine the prediction model of total flavonoid content extract of pasak bumi root.

The success of sample grouping with PCA in terms of the total variation represented and visualization of the score plot (Hermanto *et al.*, 2015). Whereas the accuracy of the prediction model for determined total flavonoid extract of Pasak

Bumi root using PLSR was by the correlation value and the RMSE value obtained. The total flavonoid prediction model can be used if the RMSEC and RMSECV values are close to 0 and the  $R^2$  value is close to 1 (Lukman, 2015).

## **III. RESULT AND DISCUSSION**

### **A. Extraction and drying of extracts**

Percentage of the yield of viscous extracts from Mandiangin, Condong, and Sabuai were 6.83%; 5.25%; and 5.22%. The viscous extract obtained in the form of viscous with a dark brown color, smells typical and tastes bitter. Measurement with FTIR-ATR using dried extract of Pasak Bumi root was intended so that the IR spectra produced only showed uptake of functional groups of compounds contained in extracts of Pasak Bumi root. The presence of solvents that are still present in the viscous extract will affect the absorption intensity in the IR spectra produced. The dried extract produced in the form of fine powder with brown color that was brighter, smells typical and tasted bitter.

### **B. Identification of Flavonoids**

The results of the identification of flavonoids carried out are as follows (Table I).

### **C. Determination of total flavonoid content with UV-Vis Spectrophotometer**

Determination of total flavonoid content using a colorimetric method that would produce a yellow color. The yellow color formed due to the formation of a complex between aluminum chloride was

a reagent in this method with ketones in C-4 atoms and hydroxyl groups in C-3 or C-5 atoms of flavones and flavonols. The total flavonoid content of extracts from three growing areas can be seen in Table II.

**Table I:** Results of identification of flavonoids.

No	Method	Result	Information
1.	1 mL of extract + sodium hidroxide + dillute hydrochloride acid	(+) (+)	Yellow solution The colour disappear
2.	1 mL extract + lead acetat 10%	(+)	Yellow sediment
3.	Filter paper + extract and evaporated with ammonia	(+)	Yellow spot

#### D. Interpretation of IR Spectra of Pasak Bumi Root Extract

The data produced in the form of absorbance in 1866 wavenumber of the pasak bumi root extract in the range 4000-400  $\text{cm}^{-1}$ . Absorption patterns appeared similar to each other and differ only in the

level of absorbance of each spectrum. Based on the spectra, the absorption peak showed the presence of O-H, C-H, = C-H, C = O, C = C and C-O groups. The presence of C = O groups is the main characteristic of flavonoid compounds (Sariningsih *et al.*, 2015).

**Table II:** Total flavonoid levels extract of pasak bumi root (standard method).

Area	Total flavonoid content (% b/b)	$\bar{x}$ Total flavonoid content (% b/b $\pm$ SD)
Mandiingin	0,223	0,224 $\pm$ 0,001
	0,224	
	0,225	
Condong	0,435	0,437 $\pm$ 0,003
	0,435	
	0,440	
Sabuai	0,459	0,467 $\pm$ 0,008
	0,467	
	0,475	

#### E. Analysis with PCA Chemometrics

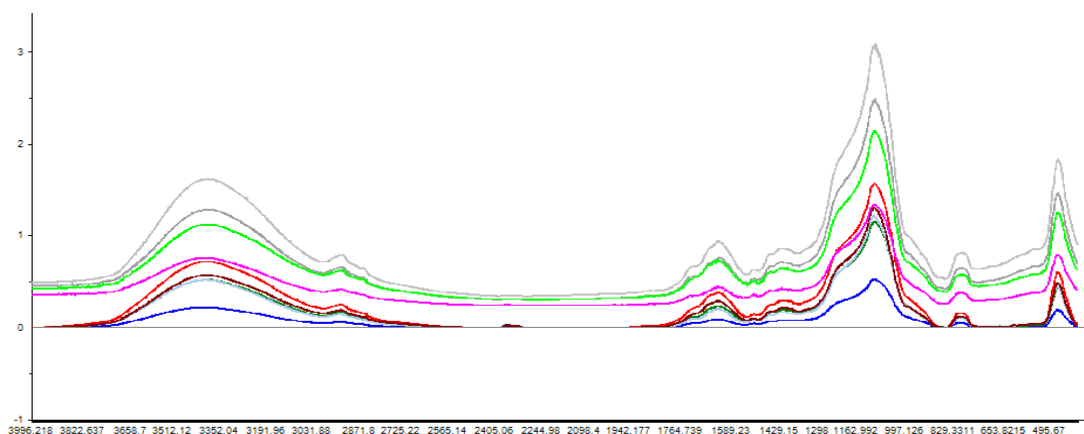
Spectra was given a preprocced of baseline correction and normalization before being used for analysis with PCA and PLSR. The preprocced IR spectra is

useful for correcting line shifts in the spectra and maximizing the resolution of overlapping data so as to increased the quality of the data analyzed (Stuart, 2004). Analysis was carried out on spectra with a

range of certain wavenumbers. The selection of wavenumber ranges was based on the presence of absorption peaks in IR spectra.

The PCA technique is useful for reducing the variables that are owned by the spectra into just a few main variables. The PCA technique will reduce the dimensions of the initial data (thousands of variables as much as wavenumbers when measuring) to only two variables. This technique will grouping samples based on

the similarity of the IR spectra they have. This grouping is based on the difference in the grow areas of samples. The results of the analysis with PCA are indicated by the score plot. The first two PCs on the score plot are the main components that are most useful in grouping because they show the most variation in data. The best score plot will clearly show the grouping of sample spectral points with the same growing areas.



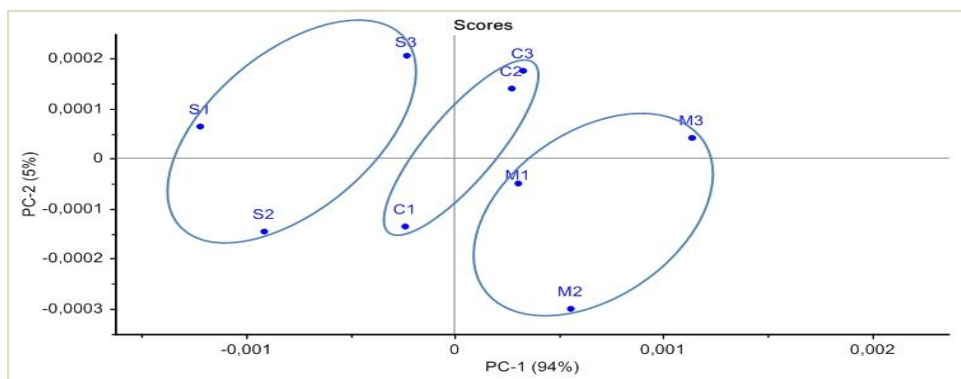
**Fig 1:** IR spectra of extracts from three growing areas.

The best IR spectra grouping was shown in the wavenumber range 1800-1540  $\text{cm}^{-1}$  with a total variation of 99% (Fig 1). This PCA score plot showed the grouping of samples based on different growth areas that show the position of the sample points sequentially based on the level of flavonoid content from the highest to the lowest, namely from Sabuai, Condong, and Mandiangin. Samples with the highest content of flavonoids (from Sabuai) were in the negative area of PC1

and positive for PC2. While the sample with the lowest flavonoid content (from Mandiangin) was in the positive area of PC1 and negative PC2. According to Kurniasari (2006), the closer the point was to another point, the greater the similarity in the nature and chemical composition of the sample. The distance between sample points in one area was still a bit far apart and shows the proximity to the sample points with other growing areas. This indicated that the pasak bumi from the

three growing areas have chemical characteristics that almost similar to each

other both in terms of their chemical characteristics and composition.



**Fig. 2 :** Plot score in the wavenumber range  $1800\text{-}1540\text{ cm}^{-1}$  from the main two PCs. (C1, C2, C3) Pasak bumi from Condong replication 1-3; (M1, M2, M3) Pasak bumi from Mandiangin replication 1-3; (S1, S2, S3) Pasak bumi from Sabuai replication 1-3.

#### F. Analysis with PLSR Chemometrics

The PLSR method aims to determine the level of linear relationship between variables  $x$  (predictors) and variables  $y$  (response). The absorbance value of the FTIR measurement was used as the variable  $x$  and the total flavonoid content measured by the standard method as variable  $y$ . Analysis with PLSR technique was carried out in several ranges of wave numbers in IR spectra. A good prediction model was indicated by a high correlation value while the error value is low. Model selection must also consider the proximity of the calibration value and validation of the correlation and error obtained. The results of the analysis with the PLSR can be seen in Table III.

The best prediction model was shown in the number range wavelength  $1800\text{-}1540\text{ cm}^{-1}$  (Fig. 2) which showed the

correlation value and close error ( $R^2$  calibration = 0.995;  $R^2$  = 0.970; RMSEC = 0.008; RMSECV = 0.021). The acceptance of model was indicated by the slope value of 0.995. According to Naes *et al.* (2002), a prediction model was to be good if it has a slope value close to 1 ( $45^\circ$ ) and the difference between the calibration and validation values was small on the correlation and the error obtained. So, this model was used to predict the total flavonoid content of extract of pasak bumi root with the equation  $y = 0.995x + 0.002$ .

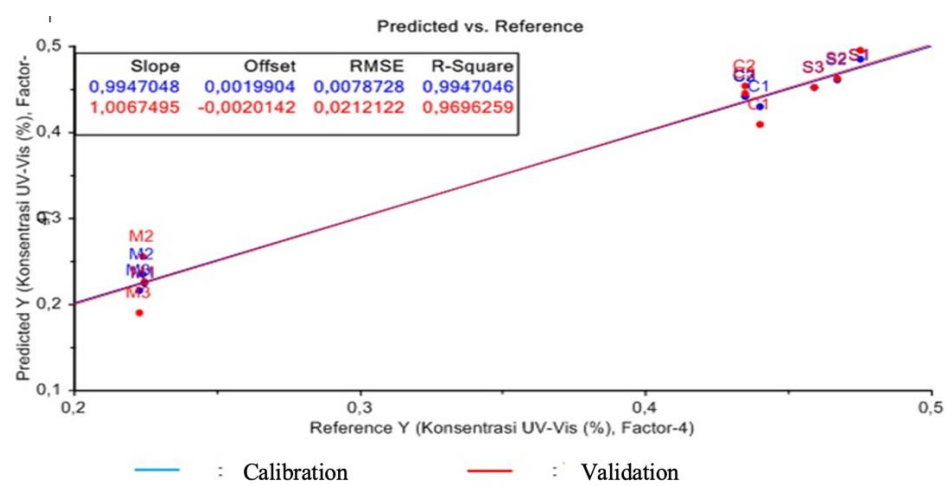
The IR spectra with selected wavenumber ranges ( $1800\text{-}1540\text{ cm}^{-1}$ ) are vibrational frequency regions of C = O ketones which are the main characteristics of flavonoid compounds (Sariningsih *et al.*, 2015). This is also supported by the presence of flavonoid compounds in the root of the pasak bumi that is 3,5,6,7,8,3',

4'-heptamethoxyflavone which has carbonyl groups in its chemical structure (Tran *et al.*, 2014). The wavenumber range chosen as the prediction model is almost the same as the study by Lukman (2015) with a wavenumber range of 1650-1400  $\text{cm}^{-1}$ .

The prediction model was used to determine the total flavonoid content of pasak bumi root. The results of the determination of total flavonoid content in the pasak bumi root can be seen in Table IV.

**Table III.** The results of the analysis with the PLSR

Wavenumber ( $\text{cm}^{-1}$ )	Correlation		RMSEC	RMSECV	Factor
	Calibration	Validation			
4000-400	0,9982454	0,7235235	0,0045318	0,0639975	6
3600-3000	0,997937	0,8023808	0,004914	0,0541064	6
3000-2800	0,9973659	0,8531471	0,0055527	0,0466418	5
<b>1800-1540</b>	<b>0,9947046</b>	<b>0,9696259</b>	<b>0,0078728</b>	<b>0,0212122</b>	<b>4</b>
870-715	0,9340395	0,8163834	0,0277858	0,0521543	3



**Fig. 3:** Plot score with PLSR on spectra with wavenumber ranges from 1800-1540  $\text{cm}^{-1}$ .

**Table IV:** Predicted values of total flavonoid content of pasak bumi root extract.

Area	Reference values	Predicted values	$\bar{x}$ prediction values (%b/b $\pm$ SD)
Mandiingin	0,223	0,216	0,225 $\pm$ 0,009
	0,224	0,235	
	0,225	0,224	
Condong	0,435	0,441	0,437 $\pm$ 0,007
	0,435	0,442	
	0,440	0,429	
Sabuai	0,459	0,452	0,466 $\pm$ 0,016
	0,467	0,461	
	0,475	0,484	



The value of the correlation coefficient (r) obtained at 0.997 showed that the predicted value of total flavonoids has 99.7% linear relationship with reference values. In addition, this model also showed good R<sup>2</sup> and RMSEP values of 0.995 and 0.008 which indicated that the model had good quality analysis results.

#### IV. CONCLUSION

The combination of FTIR and chemometrics can be used to determine the total flavonoid content of pasak bumi root extract with the calibration prediction model equation  $y = 0.995x + 0.002$  (calibration R<sup>2</sup> = 0.995; validation R<sup>2</sup> = 0.970; RMSEC = 0.008; RMSECV = 0.021).

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