

Liling_RJPBCS_1

by

Submission date: 02-May-2023 08:06PM (UTC-0400)

Submission ID: 2082533923

File name: 95_7.pdf (1.04M)

Word count: 2708

Character count: 15304



Research Journal of Pharmaceutical, Biological and Chemical Sciences

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

Liling Triyasmono^{1*}, Noor Cahaya¹, Dodon Turianto², Heri Budi Santoso³, and Abdul Rohman^{4,5}.

¹Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjar Baru 70713, Indonesia

²Department of Computer Science, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjar Baru 70713, Indonesia

³Department of Biology, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjar Baru 70713, Indonesia

⁴Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 55281 Indonesia.

⁵Research Center of Halal Products, Gadjah Mada University, Yogyakarta 55281 Indonesia.

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 chosen to develop 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 (AIC₃ 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 AIC₃ method + 0.065

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

*Corresponding author



INTRODUCTION

Essentially, flavone 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 antiinflammatory (Sutomo et al 2013, Fakhruddin 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 plants 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 allow 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 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 *Phyllanthus 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 (Darmstadt, 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 weighing 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, 1 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

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 precision 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 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.

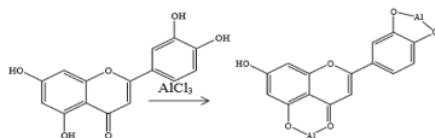


Figure 1. Reaction of complex formation with AlCl_3

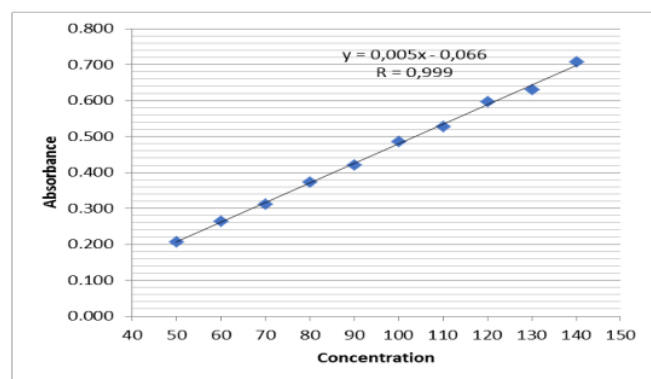


Figure 2. Standard curve of Quercetin

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.

Table 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 7000. 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, respectively which are the main group of flavonoid (Molyneux, 2010). The difference of absorption peaks was affected by the concentration of each flavonoid. The main differences of Absorption also shown at the fingerprint region. However, the subtle 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).

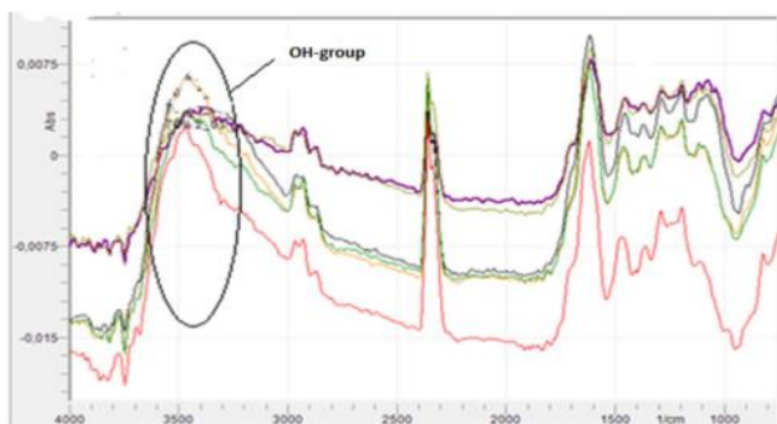


Figure 3. FTIR Spectrum of ethanolic extract of Kasturi

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 automatically the specific 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 optimization 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

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 $3300 - 3700 \text{ cm}^{-1}$. The PLS calibration model shows the coefficient determination (R^2) value was 0.998 and RMSEC value of 0.20%. The high value of R^2 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.

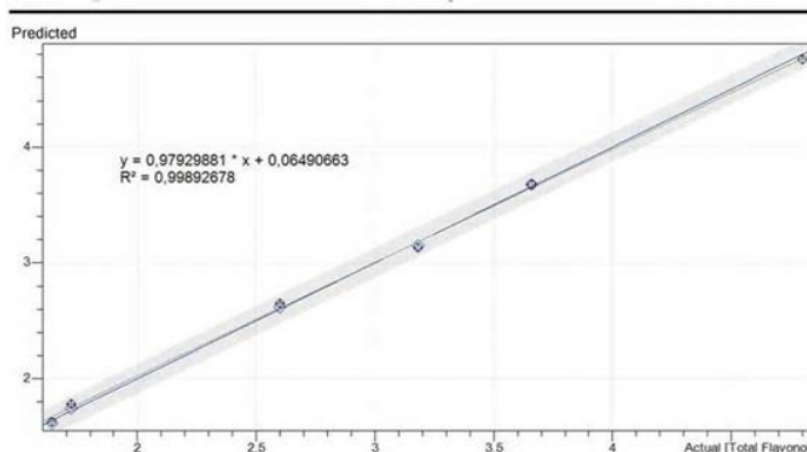


Figure 4. PLS regression plot for the relationship between of actual value (x-axis) and FTIR predicted value (y-axis) of flavonoid content in ethanolic extract of Kasturi in calibration model.

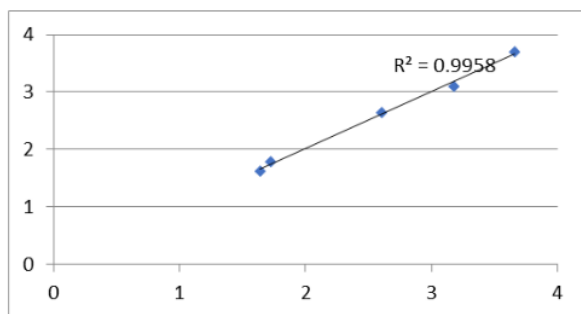


Figure 5. PLS regression plot for the relationship between of actual value (x-axis) and FTIR predicted value (y-axis) of flavonoid content in ethanolic extract of Kasturi in validation model.

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.

8

ACKNOWLEDGEMENT

The authors thank to The Ministry of Technology, Research, and Higher Education, Republic of Indonesia for the PEKERTI grant (056/UN8.2/PL/2015). The integrated Laboratory of Research and Testing was acknowledged for providing FTIR spectrophotometer.

REFERENCES

- [1] Baranska, M., Schulz, R., Siuda, R., Strehle, M.H Rosch, P., Popp, J., Joubert and E., Manly, M., 2005, Quality Control of Harpagophytum procumbens and Its Related Phytopharmaceutical Products by Means of NIR-FT Raman Spectroscopy, *Biopolymers*, 77: 1-8.
- [2] Davies, A. M. C., 1998, Cross Validation: Do We Love It Too Much?, *Spectrosc. Eur.*, 10: 24-25.
- [3] Desmiaty, Y., J. Ratnawati, & P. Andini. 2009. *Penentuan Jumlah Flavonoid Total Ekstrak Etanol Daun Buah Merah (Pandanus conoideus Lamk.) Secara Kolorimetri Komplementer*. Dipresentasikan Pada Seminar Nasional POKJANAS TOI XXXVI 13 & 14 Mei 2009, Universitas Sanata Dharma, Yogyakarta.
- [4] Devika, Mohandass, Nusrath. 2013. Fourier Transform Infra Red (FT-IR) Spectral Studies Of Foeniculum Vulgare. *Int. Res. J. Pharm.* 4(3) 203-206.
- [5] Dharmaraj, S., Jamaludin, A.M., Razak, H.M., Valliapan, R., Ahmad, N.A., and Ismail, Z.A., 2006, The classification of Phylantus niruri Linn, According to Location by Infrared Spectroscopy, *Vibrational Spectroscopy*, 41: 68-72
- [6] Diaz, T.G., Meras, I.D., Casas, J.S., Franco, M.F.A. 2005. Characterization of virgin olive oils according to its triglycerides and sterols composition by chemometric methods. *Food Control* 16: 339-347.
- [7] Fakhrudin, N, Peni Susilowati Putri, Sutomo, Subagus Wahyuono, 2013, Antiinflammatory Activity of Methanolic Extract of Mangifera casturi in Thioglycollate-Induced Leukocyte Migration on Mice, *Tarditional Medicine Journal*, Vol 18 No:3
- [8] Lisa Andina, Sugeng Riyanto and **Abdul Rohman**. 2014, Rapid determination of anisidine value of red fruit oil under elevated temperature using FTIR spectroscopy and multivariate calibration. *International Food Research Journal* 21(6): 2325-2330.
- [9] Ma, K, 2000. Peroxide value and Trans analyses by Fourier Transform Infra red (FTIR) Spectroscopy, *Disertation*, Ph.D, Department of Food Science and agriculture Chemistry, MacDonald Campus of McGill University, Montreal, Canada : 9-10, 19-20
- [10] [10] Miller, J.N., and Miller, J.C., 2010. *Statistic ad Chemometrics for Analytical Chemistry*, 6th edition, Prentice Hall, England, p 110-111, 243-244.
- [11] Pavia DL, Lampman GM, Kris GS. 2009. *Introduction to Spectroscopy*. 4th edition. Washington DC: Thomson Learning Brooks/Cole.
- [12] Rohman, A., Che Man, Y.B., and Riyanto, S. 2011, Authentication analysis of red fruit (*Pandanus Conoideus*, Lam) oil using FTIR spectroscopy in combination with chemometrics, *Phytochem Anal* 22 (5):462-467.
- [13] Rohman, A., Nugroho, A., Lukitaningsih, E. and Sudjadi. 2014. Application of vibrational spectroscopy in combination with chemometrics techniques for authentication of herbal medicine. *Applied Spectroscopy Reviews*. 49:8, 603-613
- [14] Sutomo, S. Wahyuono, S. Rianto and E.P. Setyowati, 2013. Isolation and Identification of Active Compound of n-hexane Fraction from Kasturi (*Mangifera casturi* Konsterm.) against Antioxidant and Immunomodulatory Activity. *Journal of Biological Sciences*, 13: 596-604. Stuart, B. 2004, *Infra Red Spectroscopy: Fundamental and Application*, p.18-34. John Wiley & Sons, Ltd.
- [15] Triyasmono, L., Riyanto, s., Rohman, A., 2013, Determination of Iodine value and Acid Value of Red Fruit Oil by Infrared Spectroscopy and Multivariate Calibration, *Int. Food and Reseach J*, 20(6): 3259-3263
- [16] Van de Voort, F.R., Sedman, J., Emo, G., and Ismail, A.A. 1992. Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *Journal of the American Oil Chemists' Society* 69(11):1118-1123.

ORIGINALITY REPORT

16%

SIMILARITY INDEX

12%

INTERNET SOURCES

10%

PUBLICATIONS

%

STUDENT PAPERS

PRIMARY SOURCES

1	innovareacademics.in Internet Source	3%
2	Liling Triyasmono, Curd Schollmayer, Jens Schmitz, Emilie Hovah, Cristian Lombo, Sebastian Schmidt, Ulrike Holzgrabe. "Simultaneous Determination of the Saponification Value, Acid Value, Ester Value, and Iodine Value in Commercially Available Red Fruit Oil (Pandanus conoideus, Lam.) Using ¹ H qNMR Spectroscopy", Food Analytical Methods, 2022 Publication	2%
3	www.science.gov Internet Source	1%
4	academicjournals.org Internet Source	1%
5	journal.uad.ac.id Internet Source	1%
6	mymedr.afpm.org.my Internet Source	1%

7	agris.fao.org Internet Source	1 %
8	media.neliti.com Internet Source	1 %
9	journals.utm.my Internet Source	<1 %
10	Abdul Rohman, Ardi Nugroho, Endang Lukitaningsih, Sudjadi. "Application of Vibrational Spectroscopy in Combination with Chemometrics Techniques for Authentication of Herbal Medicine", <i>Applied Spectroscopy Reviews</i> , 2014 Publication	<1 %
11	Lanruo Zhao, Hongduo Zhao, Juewei Cai. "Tire-pavement friction modeling considering pavement texture and water film", <i>International Journal of Transportation Science and Technology</i> , 2023 Publication	<1 %
12	Ozlem Bakir Boga, Ahmet Bugra Ortaakarsu, Beyzanur Kadirustaoglu, Esabi Basaran Kurbanoglu. " Phytochemical Profiling, Biological Activities and Molecular Docking Studies of the Crude Extract of , an Endemic Plant in Turkey ", <i>Chemistry & Biodiversity</i> , 2023 Publication	<1 %

13	biblio.vub.ac.be Internet Source	<1 %
14	"Application of near- and mid-infrared spectroscopy combined with chemometrics for discrimination and authentication of herbal products: A review", Journal of Applied Pharmaceutical Science, 2019 Publication	<1 %
15	Isaac Kingsley Amponsah, Abena Boakye, Emmanuel Orman, Francis Ackah Armah et al. "Assessment of some quality parameters and chemometric-assisted FTIR spectral analysis of commercial powdered ginger products on the Ghanaian market", Heliyon, 2022 Publication	<1 %
16	ejournal2.litbang.kemkes.go.id Internet Source	<1 %
17	ojs.uho.ac.id Internet Source	<1 %
18	psasir.upm.edu.my Internet Source	<1 %
19	www.japsonline.com Internet Source	<1 %
20	Abate Limenew, Mengistu Tadesse. "Phytochemical screening and peroxide value determination of methanolic extract of four	<1 %

traditional medicinal plants from Debre Tabor
Town, Ethiopia", Journal of Medicinal Plants
Research, 2018

Publication

21

Bahman Nickavar, Mohammad Kamalinejad,
Maher Haj-Yahya, Bijan Shafaghi. "

Comparison of the Free Radical Scavenging
Activity of Six Iranian . Species ",
Pharmaceutical Biology, 2008

Publication

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On