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IAI SPECIAL EDITION

RESEARCH ARTICLE

Infrared spectroscopy chemometric model for determination of phenolic content of plant leaf powder

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Keywords
Infrared spectroscopy
Chemometrics
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Plant leaf powder

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Abstract

Introduction: The leaf is the part of the plant often used in traditional medicine because it is rich in one class of secondary metabolite compounds, namely phenolic compounds. **Objectives:** This study aims to establish a chemometric model for determining the phenolic content of plant leaf powders using the infrared spectroscopy (FTIR and NIR) method with a combination of chemometrics. **Methods:** The dried and powdered plant leaves were scanned using FTIR and NIR spectroscopy. Spectra were used to form calibration models. The calibration models were Partial Least Square (PLS), Principal Component Regression (PCR), and Support Vector Regression (SVR). The selected calibration model was validated using LOOCV and external cross-validation. The best calibration model was PCR, with R^2 and RMSEC values of FTIR and NIR of 0.9918885; 0.9752648 and 0.8675906; 1.5150245, respectively. **Results:** The results of the Paired-Sample T-test analysis of actual samples determined by the selected calibration model compared to the comparison method showed no significant difference.

Introduction

The leaf is the part of the plant often used in traditional medicine. It is abundantly available in nature and is easy to use and retrieve (Jadid *et al.,* 2020). One of the secondary metabolites found in leaves is phenolic compounds. Phenolic compounds have one or more hydroxyl groups attached to an aromatic ring and are formed from the shikimic acid metabolic pathway. Phenolic compounds are secondary metabolites primarily distributed in plants and essential for growth (Bhatla & Lal, 2018). Additionally, phenolic compounds have antioxidant, antiinflammatory, antiproliferative, antimutagenic, antimicrobial, anticarcinogenic, and cardioprotective properties (Akhtar *et al.,* 2019).

Methods

In this study, the determination of the total phenolic content in leaf plants was done with UV-Vis spectroscopy and Folin-Ciocalteu's reagent. Gallic acid used as the standard served to compare the total phenolic content (Blainski *et al.,* 2013). This study also used the Fourier Transform InfraRed (FTIR) and Near InfraRed (NIR) spectroscopy coupled with chemometric analysis. Infrared spectroscopy is an effective analytical technique because it is non-destructive, uses simple preparation, does not require chemicals, and analyses quickly (Haas & Mizaikoff, 2016). However, the spectra generated from FTIR and NIR spectrophotometers are very complicated and overlapping.

Chemometrics were used to assist in analysing the spectral data generated from these two instruments. The authors had previously developed a method to determine the total phenol in plant leaf extracts using IR spectroscopy and chemometrics (Wulandari *et al.,* 2020). This method will be applied in this study on plant leaf powder.

Materials

Twenty-five (25) leaf samples of medicinal plants with

varieties of phenolic content were collected from the residential and plantation areas in Jember city, East Java, Indonesia (Table I). Reagents used were ethanol 96%, gallic acid (Sigma-Aldrich), Folin-Ciocalteu's reagent (Merck), Na_2CO_3 (Merck), filter paper, and distilled aqua dest. Five commercial samples (capsule preparations) were purchased from a pharmacy department store in Jember city. The instruments used were Moisture Analyser type PMB 53, NIR spectroscopy (Brimrose Corporation Luminar 3070), FTIR spectroscopy (Bruker Alpha), and UV-Vis spectroscopy (Hitachi U 1800).

Table I: Samples of leaves used and total phenolic content of the samples

No	Training set sample		mg GAE/g powder ± RSD (%)
	Code	Name	
1	A	*Carica papaya*	14.43 ± 3.520
2	B	*Moringa oleifera*	28.33 ± 1.547
3	C	*Averrhoa Carambola*	29.60 ± 3.056
4	D	*Ocimum basilicum*	31.83 ± 1.248
5	E	*Gnetum gnemon*	33.31 ± 1.201
6	G	*Pandanus amaryllifolius*	34.50 ± 0.785
7	H	*Leucaena leucocephala*	34.59 ± 0.994
8	I	*Artocarpus heterophyllous*	35.19 ± 0.754
9	J	*Morinda citrifolia*	35.57 ± 1.817
10	L	*Diplazium esculentum*	37.08 ± 2.410
11	N	*Syzgium aqueum*	39.06 ± 1.643
12	O	*Annona muricata*	39.42 ± 2.630
13	Q	*Psidium guajava*	41.56 ± 1.770
14	R	*Sauropus androgynous*	45.19 ± 1.741
15	T	*Anredera cordifilia*	45.98 ± 0.833
16	U	*Syzygium polyanthum*	47.19 ± 0.873
17	V	*Cosmos caudatus*	47.31 ± 0.333
18	W	*Nephelium lappaceum*	50.28 ± 1.294
19	Y	*Persea Americana*	60.27 ± 0.527
20	F	*Piper betle*	34.12 ± 1.752
21	K	*Coffea canephora*	36.89 ± 1.598
22	M	*Chrysophyllum cainito*	38.20 ± 2.418
23	P	*Pluchea indica*	40.47 ± 3.556
24	S	*Pometia pinnata*	45.27 ± 1.608
25	X	*Mangifera indica*	55.34 ± 1.879

Sample preparation

Leaf samples were powdered by a blender and divided into two groups, the training set and the test set. All samples were dried to fulfil the requirement of the moisture content of herbal powder that is below 10%.

Determination of the total phenolic content by UV-Vis spectroscopy as a comparing method

For the standard solution, 25 mg and 50 mg of gallic acid were weighed and dissolved in ethanol 96%, then diluted to concentrations of 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm, 140 ppm, and 200 ppm. Then, 100.0 µl of each standard and sample solution was pipetted and added with 500.00 µl of Folin-Ciocalteu's reagent, left for six minutes, added with 400.0 µl of Na_2CO_3 at 7.5 %, then allowed to stand for 80 minutes. The mixed solution was measured at a wavelength of 743 nm.

Determination of FTIR and NIR Spectra Data

All samples and gallic acid standards were scanned five replications by placing difference powder in the sample compartment of NIR and FTIR spectrometers. Each scan had five shots (Rahmawati *et al.,* 2015; Wulandari *et al.,* 2016). The NIR and FTIR spectra data were obtained through Acquire Brimrose (NIR) and OPUS (FTIR) software and each spectra data were code-named.

Determination of model calibration and validation of the model

Spectral data from FTIR at 4000–650 cm^{-1} and NIR at 850–2000 nm were analysed by chemometrics using the Unscrambler X software version 10.2. Partial Least Square (PLS), Principal Component Regression (PCR), and Support Vector Regression (SVR) analysed FTIR and NIR spectral data to form a calibration model, and then the best model was selected based on R^2 value close to

one and the smallest value of RMSEC (Root Mean Square of Calibration) and RMSECV (Root Mean Square Error of Cross-Validation). The chosen model was validated using leave one out cross-validation (LOOCV) by taking out one of the training set sample data. The remaining data were used to reform the model. For external cross-validation, the test set consisting of six independent samples was analysed by the model, and the accuracy data results were determined. After validation, the model could be applied to determine the phenolic content of actual samples on the market. The result of the actual sample analysis was compared with the reference method (UV-vis spectrophotometry) with paired sample T-test (Kumar *et al.,* 2015; Nicenboim & Vasishth, 2016).

Results

Each sample was provided with an identity code. The moisture content of all samples was less than 10% to eliminate the interference of water spectra and prevent microbial growth. The water content of the training set and the sample test set ranged from 3-6%. FTIR spectra of sample and gallic acid showed similar profiles with different intensities of reflectance (Figure 1). The results of total phenolic levels from the training set and the sample test set can be seen in Table I. The results obtained by the comparison method are in the range of 14.43-60.27 mg GAE/g.

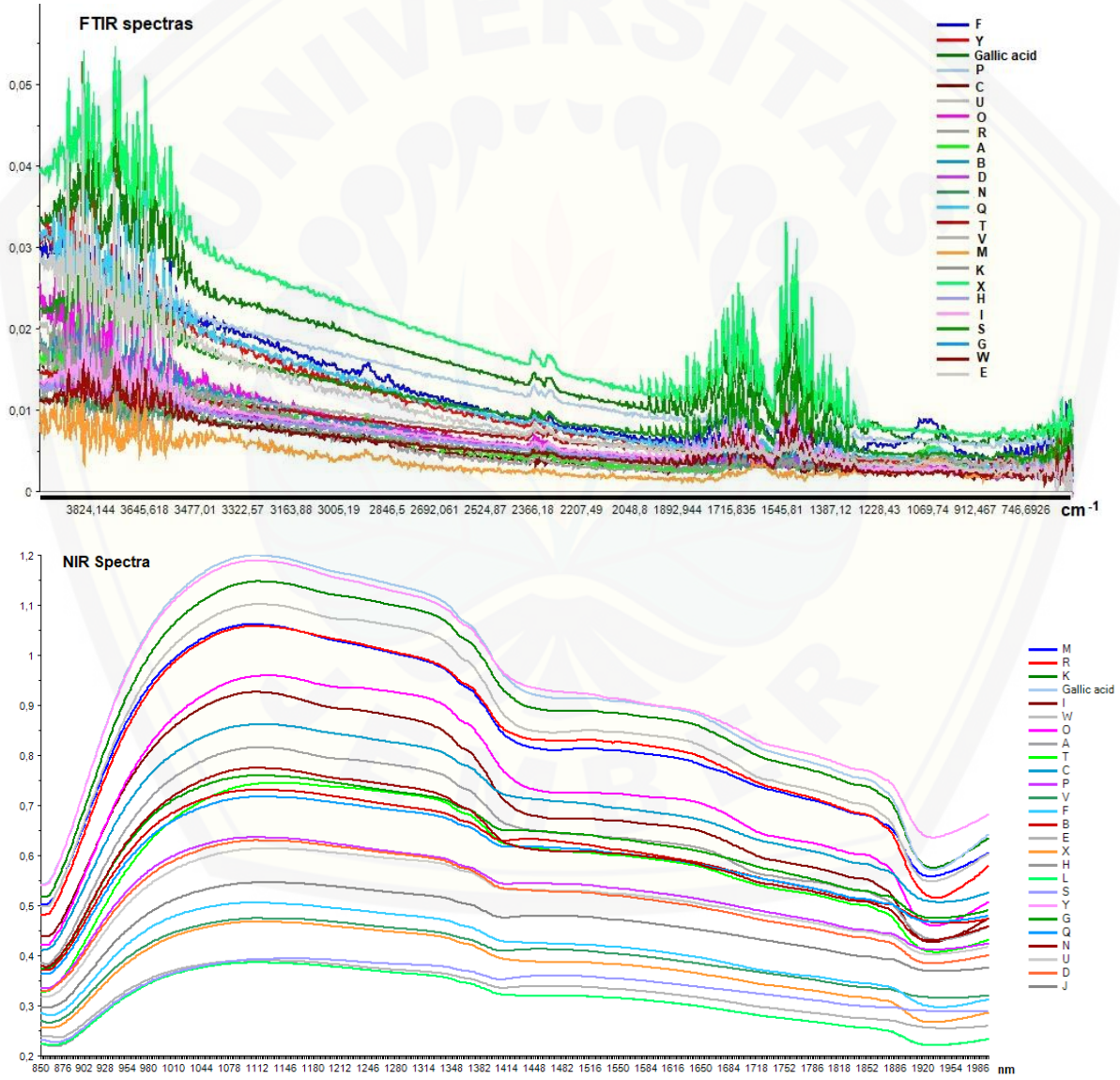

Figure 1: FTIR and NIR spectra of samples and gallic acid

The formation of the calibration model carried out on the training set data was obtained from FTIR and NIR spectra, processed, and developed for a calibration model with chemometrics. Table II shows that the best calibration model of FTIR spectra was PCR, with R^2, RMSE (Root Mean Square Error), and RMSECV values of 0.992, 0.868, and 0.980, respectively. The best calibration model of NIR spectra was also PCR, with R^2, RMSE, and RMSECV values of 0.975, 1.515, and 1.577, respectively. The results of the cross-validation showed that the selected model (PCR) was related to infrared spectra and phenolic content based on the R^2 result of LOOCV, higher than 0.91, with RMSE (Root Mean Square Error) and RMSECV having already small values (Lengkey *et al.,* 2013). The result of the external cross-validation that was carried out using independent samples from a test set of known groups (Jung & Hu, 2015) showed valid results with an R^2 value of 0.989 and an RMSE value of 0.748, indicating that the selected model has good reliability. The selected and validated model was applied to determine the phenolic content in five actual samples as described in Table III. Table III shows that the total actual phenolic content measured by the FTIR-NIR chemometric model and the reference method (UV-vis spectroscopy) had no significant difference (p>0.05) (Santoso, 2014).

Table II: Calibration results of the chemometric model

Spectra	Model	RMSE	R-Square
FTIR	PLS		
	Calibration	1.079	0.987
	Validation	1.192	0.985
	PCR		
	Calibration	0.868	0.992
	Validation	0.980	0.990
	SVR		
	Calibration	1.837	0.967
	Validation	1.837	0.967
Spectra	**Model**	**RMSE**	**R-Square**
NIR	PLS		
	Calibration	1.638	0.971
	Validation	1.702	0.969
	PCR		
	Calibration	1.515	0.975
	Validation	1.577	0.973
	SVR		
	Calibration	2.334	0.943
	Validation	2.438	0.937

Table III: Results of total phenolic levels in actual samples

Real Sample	mg GAE/ g powder ± RSD (%)		
	FTIR	NIR	UV-Vis
SN 1	25.06 ± 0.028	24.94 ± 0.064	25.15 ± 1.569
SN2	38.06 ± 0.015	39.13 ± 0.360	39.26 ± 2.927
SN3	21.55 ± 0.039	21.77 ± 0.160	21.74 ± 3.049
SN4	40.65 ± 0.020	40.46 ± 0.200	40.49 ± 1.903
SN5	15.84 ± 0.018	16.02 ± 0.890	16.03 ± 1.338

Discussion

A total of 25 samples were used, divided into 19 training sets and six sample test sets. The standard used was gallic acid because of its three hydroxyl groups (the more the hydroxyl groups, the higher the antioxidant activity) and because it is a simple phenolic derivative (Fernandes & Salgado, 2016). In this study, the Folin-Ciocalteu's reagent was used to measure phenolic compounds in the test sample by colourimetric oxidation and reduction reaction. This reagent can oxidise phenolic compounds and reduce heteropoly acid to a molybdenum-tungsten complex (Hudz *et al.,* 2019). The addition of 7.5% Na_2CO_3 made the medium alkaline because phenolic compounds can only react

with the Folin-Ciocalteu's reagent under alkaline conditions. The dark blue colour indicated the higher concentrations of phenolic compounds. More phenolic ions reduce heteropoly acids to detect them later by UV-Vis spectroscopy (Asrin *et al.,* 2018). The calibration model showed good results with R^2 closer to one and a small RMSE value. RMSEC and RMSECV were based on the smallest value. R^2 was close to one, indicating a linear correlation between the response variable and the predictor variable. The smaller the RMSE, the smaller the error of the model in predicting the response (Georgieva *et al.,* 2013).

Conclusion

The infrared spectroscopy chemometric model can be used to determine the phenolic content of plant leaf powders. This method is simple, accurate, and environmentally friendly.

Reference

Akhtar, M.S., Swamy, M.K., & Sinniah, U.R. (2019). Natural Bio-Active Compounds Volume 1: Production and Applications. Springer Nature Singapore

Asrin, H., Hasibuan, P.A.Z., & Marianne, M. (2018). Total Phenolic Content of Ethanol Extract of Artrocarpus camansi Leave and its Effect to SOD (Superoxide Dismutase) Level in Mice. *Indonesian Journal of Cancer Chemoprevention,* **8**(3), 101-109. http://dx.doi.org/10.14499/indonesianjcanchemoprev8iss3pp101-109

Bhatla, S. & Lal. M.A. (2018). Plant Physiology, Development and Metabolism. Springer Nature Singapore Pte Ltd

Blainski, A., Lopes, G.C. & De Mello, J.C.P. (2013). Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from limonium brasiliense l. *Molecules*. **18**(6):6852–6865. http://doi10.3390/molecules18066852

Fernandes, F.H.A & Salgado, H.R.N. (2016). Gallic acid: review of the methods of determination and quantification. *Critical Reviews in Analytical Chemistry.* **46**(3):257-265 http:/doi:10.1080/10408347.2015.1095064

Georgieva, M., Nebojan, I., Mihalev, K., Yoncheva, N., Kljusuric, J.G., & Kurtanjek, Z. (2013). Application of NIR spectroscopy and chemometric in quallity control of wild berry fruit extracts during storage. *Croatian journal of food technology, biotechnology and nutrition*, **8**(3-4), 67-73

Haas, J. & Mizaikoff, B. (2016). Advances in mid-infrared spectroscopy for chemical analysis. *Annual review of analytical chemistry (Palo Alto, Calif.)*, **9**(1), 45–68. https://doi.org/10.1146/annurev-anchem-071015-041507

Hudz, N., Yezerska, O., Shanaida, M., Sedlackova, V.H. & Wieczorek, P.P. (2019). Application of the folin-ciocalteu method to the evaluation of salvia sclarea extracts. *Pharmacia,* **66**(4):209-215 http:/doi.10.3897/pharmacia.66.e38976

Jadid, N., Kurniawan, E., Himayani, C.E.S., Andriyani, Prasetyowati, I., Purwani, K. I., & Tjahjaningrum, I.T.D. (2020). An ethnobotanical study of medical plants used by the Tengger tribe in Ngadisari village, Indonesia. *PLOS ONE.* **15**(7) 1-16 http:/doi:10.1371/journal.pone.0235886

Jung, Y. & Hu, J. (2015). A k-fold averaging cross-validation procedure. *Journal of Nonparametric Statistics.* **27**(2):167–179. http:/doi:10.1080/10485252.2015.1010532

Kumar, R., Kumar, V. & Sharma, V. (2015). Discrimination of various paper types using diffuse reflectance ultraviolet-visible near-infrared (uv-vis-nir) spectroscopy:forensic application to questioned documents. *Applied Spectroscopy,* **69**(6):714-720 http:/doi:10.1366/14-07663

Lengkey, L. C. E. C. ; Budiastra, W., Seminar, K. B. & Purwoko, B. S. (2013). Determination of chemical properties in jatropha curcas l. seed IP-3p by partial least-square regression and near-infrared reflectance spectroscopy. *International Journal of Agriculture Innovations and Research,* **2**(1):41–48. https://doi.org/10.17660/ActaHortic.2013.1011.42

Niceboim, B. & Vasishth, S. (2016). Statistical methods for linguistic research. *Linguistics and Language Compass.* **8**(11): 591-631 https://doi.10.1111/lnc3.12201

Rahmawati A., Kuswandi B., & Retnaningtyas Y. (2015). Detection of Porcine Gelatin in Jelly Soft Candy Sample Using Fourier Transform Infra Red and Chemometrics. *Jurnal Pustaka Kesehatan,* **3**(2), 278–283

Santoso, S. (2014). Panduan Lengkap SPSS Versi 20 Edisi. Jakarta: PT Elex Media Komputindo

Wulandari, L., Retnaningtyas, Y. & Lukman, H. (2016). Analysis of flavonoids in medicinal plant extract using infrared spectroscopy and chemometrics. *Journal of Analytical Methods in Chemistry.* **2016**:1–6 https://doi.org/10.1155/2016/4696803

Wulandari, L., Kristiningrum, N., & Ratnasari, F.A. (2020). Rapid Determination of Total Phenol in Leaf Extracts of a Medicinal Plant using Infrared Spectroscopy and Chemometric Methods. *Journal of Analytical Chemistry,* **75**(4), 479-486. https://doi.org/10.1134/S1061934820040176