

Exploring the Potential of NIR Spectroscopy and Chemometrics to Verify the Flavonoid Content in *Litsea cubeba*

Khrisna Agung Cendekiawan^{1,2}, Dhina Ayu Susanti², Nafisah Isnawati², Shinta Mayasari², Florentinus Dika Octa Riswanto^{1*}

¹ Division of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Sanata Dharma University, Campus 3 Paingen Maguwoharjo Depok Sleman, Yogyakarta 55282, Indonesia

² Pharmacy Study Program, Universitas dr. Soebandi, Jalan dr. Soebandi Nomor 99 Jember, East Java, Indonesia

ABSTRACT

The ancient records about herbal medicine show traditional healing practices are deeply rooted in Indonesian culture. The medical use of plants decreased because of inheritance customs combined with the lack of written documentation on medicinal plants but dependent on oral information transmission. Traditional research and preservation activities of local healing plants which belong to specific cultural communities become possible through this phenomenon. Researchers conducted ethnopharmacological research about antidiarrheal medicinal plant uses that included traditional knowledge about flavonoid-containing plants within the Osing ethnic group of West Java Indonesia. The goal of this research was to verify flavonoid compounds in Osing's medicinal plants through Near Infra-Red (NIR) spectroscopy combined with chemometrics techniques. The evaluation of antidiarrheal potential for these compounds relied on flavonoid standard reference substances because these compounds demonstrate strong antioxidant properties which stabilize intestinal functioning. Chemometrics techniques together with Near Infra-Red spectroscopy (NIR) were applied to analyze krangean leaves (*Litsea cubeba*) in order to establish their flavonoid compound levels. The quality assessment of multivariate calibration models shows that PLS regression produces RMSEC of 2.870 and RMSEP of 0.0124 with $R^2_{cal} = 0.9931$ and $R^2_{val} = 0.9919$. The analysis using linear discriminant analysis (LDA) succeeded in its completion with recognition rates above 90% for each discriminating class.

Keywords: chemometrics; ethnopharmacy; krangean; NIR spectroscopy; Osing

INTRODUCTION

Indonesian medicinal plants are a distinctive element of the country's apparent memory of cultural history about health, and as such they are a unique cultural legacy that possesses its therapeutic capabilities. Thus, these medicinal plants are regularly utilized by the Indonesian people in accordance with their traditional medicine benefit for the community since they have been passed down in the generations (Reyes-Garcia, 2010). Many scholars are interested in the Osing region because it stands between the Ijen Mountain and Banyuwangi Regency in East Java Province (Prasetyo et al., 2018). Although the academic studies have devoted many years in studying the religious rites based on medicinal plants being practiced among the Osing people (Kusumo et al., 2023); apparently, there is little to no documentation on the basics of knowledge pertaining to the applications and use of medicinal plants. The authors note film in Sumarni et al. (2019) as an important resource

because it facilitates key advances to help devise new treatments.

Ethnopharmacy is one tool that can provide a knowledge of how indigenous communities have used botanicals in the past for medical use. Ethnopharmacy is a field of research on traditional medicinal plant practices within the framework of traditional medicine of a given culture (Bhagawan et al., 2022). Little academic work is done on the Osing community, despite the large amount of experimental research done in ethnopharmacy in general, and in particular to Indonesia's many ethnic groups. Thus, this is the first study that aims to perform investigation to explain the traditional healing methods of the Osing ethnic group of East Java. In this way, it thus constitutes an important basis for further academic studies (Puspitasari et al., 2021). Osing tribe grows krangean leaves (*Litsea cubeba*) that are used to get rid of diarrhea in the community. Nevertheless, there have been very little collection of data on use of krangean leaves or identifying compound content which still needs to be explored.

This can be done by the use of chemometrics and Near Infra-Red (NIR) spectroscopy to predict

*Corresponding author : Florentinus Dika Octa Riswanto
Email : dikaocta@usd.ac.id

whether there were active compounds (Fakayode et al., 2020). NIR spectroscopy is the method by means of which from the electromagnetic wavelengths that lie outside of the visible light spectrum, one can determine the presence of substances in the allocated or the preparation (Hair et al., 2021). It has proven to be very useful in the study of organic substances, serving as an excellent 'tag' and extender to the electrode. Furthermore, chemometrics techniques can be performed on quantitative analysis resulting to further investigation of the differences among sample component parts, and the compound amounts can then be accurately measured (Windarsih et al., 2021). As stated by Gad et al. (2013), in order to validate medicinal plants, ensure they are safe for consumers to consume, and maximise the therapeutic benefits that are predicted. This research aimed to learn more about the ethnopharmacology of krangean (leaf) plant, which the Osing people use in treatment of antidiarrheal. This ethnopharmacological study will also serve to increase the knowledge of medicinal plant usage of the Osing tribal people, and may subsequently promote attention to the potential plant's therapeutic properties that will help lead to creating a new form of alternative medicinal practice that takes advantage of local resources. The chemometrics techniques and near infrared spectroscopy were used in the study approach as well.

MATERIALS AND METHODS

Material

A quercetin flavonoid standard, supplied from PT. Dexa Medica and validated by ChromaDex, together with samples of medicinal plants from the Osing Tribe, and krangean leaves, which included dried herbal material and reference components, were all used in the research.

Tools

The survey phase commenced with the development of a questionnaire, which underwent ethical review. An analytical balance, oven, blender, and B-30-sized sieve were among the instruments used in this study. Utilising a *Brimrose Corporation* Luminar 3070 near-infrared spectrometer (Brimrose Corporation of America, Sparks, Maryland, USA), *BRIMROSE* software, and *The Unscrambler X 10.2* software (Camo Analytics, Oslo, Norway) for data processing, the data collecting procedure was carried out.

Ethnopharmacy study

Data was collected in the Licin Subdistrict of Banyuwangi Regency using an ethnopharmacy method, after an initial survey (Ethical Approval No. 441/KEPK/UDS/VIII/2023). Because of the large Osing Tribe's presence in the region, this particular town was chosen. In the Chemistry Laboratory of the University of Jember's Faculty of Pharmacy, the collected samples will thereafter be analysed chemically. Banjar Village, Licin Subdistrict, Banyuwangi Regency (Apex: -8.193077160373061, Base: 114.26683913916348, R748+QPM) was the site of the ethnopharmacy investigation. The next step in gathering information was to administer questionnaires and conduct interviews with 96 members of the Osing Tribe residing in Banjar Village.

Sample preparation

Mixing simplicia with quercetin compounds was part of the sample preparation process. Combinations of plant simplicia and chemicals made up the final virtual samples. After a certain amount of time in the oven, the simplicia plant was removed. The next step was to crush it using a pestle and mortar so it would be ready for examination. A training set and a test set were created from the simulated samples. As shown in Table I, mixtures of quercetin flavonoid and krangean dry leaf simplicia were prepared at varying concentrations, starting from 1% and going up to 100% of the total (± 10 g).

Pure krangean dry leaf raw material, quercetin flavonoid material, and mixes of krangean dry leaf raw material at concentrations of 20%, 50%, and 70% make up the five variants of simulated krangean dry leaf herbal material data that are given in Table II. One component of the mixed krangean leaf herbal material used to evaluate the model is pure krangean dry leaf herbal material.

Chemometrics techniques

Placing the sample on the Integrating Sphere unit's holder allowed for accurate measurement. The light from the halogen bulb was directed through a series of filters designed to meet the exacting standards. When the sensor reaches the sample, it digitises the reflection of near-infrared light. With a data collection interval of 5 nm and a filter spanning a wavelength range of 1400–2000 nm, the NIR Spectrophotometer measured 120 points of reflection.

Table I. The Training Set data was developed using a mixture of krangean dry leaf simplicial

Quercetin Flavonoid (g)	Kranglean Leave Herbal Material (g)	Concentration (%)	Category
0	10	0%	Pure
0.012	10	1.20%	Mixture
0.021	10	2.10%	Mixture
0.031	10	3.10%	Mixture
0.041	10	4.10%	Mixture
0.051	10	5.10%	Mixture
0.060	10	6.00%	Mixture
0.072	10	7.20%	Mixture
0.082	10	8.20%	Mixture
0.092	10	9.20%	Mixture
0.101	10	10.05%	Mixture
0.204	10	20.41%	Mixture
0.302	10	30.18%	Mixture
0.400	10	40.03%	Mixture
0.501	10	50.10%	Mixture
0.601	10	60.05%	Mixture
0.703	10	70.28%	Mixture
0.801	10	80.12%	Mixture
0.903	10	90.33%	Mixture
1.001	10	100.11%	Mixture

Table II. Test the data set containing a blend of krangean dry leaf herbal substance and quercetin flavonoid.

Quercetin in grams	Kranglean Leaves in grams	% Concentration	Category
0	10	0%	Pure
0.204	10	20.40%	Mixture
0.501	10	50.14%	Mixture
0.703	10	70.30%	Mixture
1.001	0	100.11%	Mixture

The chemometrics study was conducted using The Unscrambler X 10.2 software (Camo Analytics, Oslo, Norway; available at <https://www.camo.com/unscramblerx/>). The most effective prediction and recognition capabilities were utilized to select the best classification models. The chemometrics model was evaluated for its predictive power using the following metrics: R^2_{cal} , R^2_{val} , RMSEC, and RMSEP, employing the Partial Least Squares (PLS) calibration function. The recognition capabilities of a model refer to how well it performs in testing on samples drawn from a training set. The prediction power of the model, on the other hand, when compared to the test set samples using Linear Discriminant Analysis (LDA), is defined as the percentage of correct classifications.

RESULTS

The Osing people make extensive use of krangean leaves to treat diarrhoea, according to the ethnopharmacological study. Additional phytochemical testing confirmed that the extract

contained quercetin flavonoids (Sumarni, 2019). Table III displays the entire findings.

In order to generate multivariate calibration models and choose the optimal number of variables, PLS was used. In addition, the LDA model that was utilised to distinguish between groups was constructed using the variable number. So, it's safe to say that the PLS can represent the connection between the independent and dependent variables, which in turn improves the accuracy of the LDA. Several features were obtained for model assessment, including R^2_{cal} , R^2_{val} , RMSEC, and RMSEP, because the PLS regression was constructed in this work. The mapping results demonstrate a distinct segregation between the categories of pure and mixed krangean dried leaf herbal material on dried leaf (Figure 1) and dried powder leaf of krangean (Figure 2).

A classification model was trained using a dataset. A chemometrics classification model was created using the spectrum data from each dataset; this model made use of Linear Discriminant

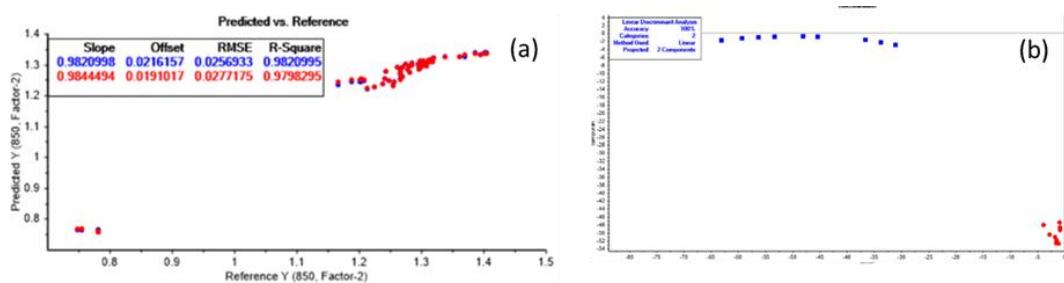


Figure 1. The Characteristics of the dried krangean leaf herbal sample training set are shown in (a) partial least squares (PLS) with R-Squared values and (b) Linear Discriminant Analysis (LDA) along with accuracy values.

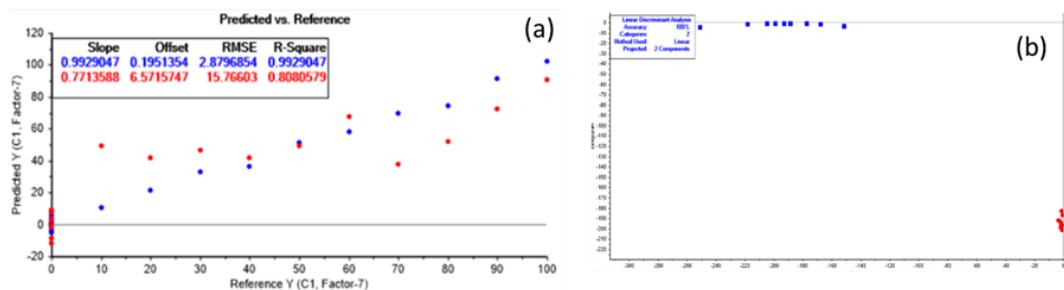


Figure 2. The characteristics of the dried krangean leaf powder herbal material training set are analyzed in (a) partial least squares (PLS) using R-Squared values and (b) Linear Discriminant Analysis (LDA) employing accuracy metrics.

Table III. Phytochemical Screening Analysis of Krangean Leaf Extract was was carried out using various tests that relied on specific reagents to identify the suspected compound content.

No	Metabolite secondary	Raw Simplicia	Dry Powder Simplicia
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Saponin	+	+
4.	Tanins	+	+
5.	Glikosid	+	+
6.	Steroid/Triterpenoid	+	+

Analysis (LDA) in particular (Luo et al., 2013). When looking at the infrared scan results in Figure 1, it's clear that the absorption patterns were similar in the training and test sets for the pure krangean leaf herbal material and the mixtures as well. The only difference was in the quantitative values of the spectra's absorbances. Because different compounds have different distinctive spectra, similar absorption patterns may point to the presence of different compounds, necessitating the determination of an acceptable spectral value. A graphic illustrating the difference between pure krangean leaf herbal material and its mixtures was given in the findings. Concurrently, the categorisation of samples into the specified categories was emphasised in the prediction table.

After that, a classification model was built utilising the training set's data. In order to create a

chemometrics classification model, we used the spectra from all of the training datasets and used Linear Discriminant Analysis (LDA). Consequently, a comprehensive evaluation was conducted to find the model that best determines a substantial association between categories and discriminants. The results of the Linear Discriminant Analysis were used to determine the percentages of recognition and prediction for each model that was built using the properties of the consumed krangean leaves. This was done, in particular, for the powdered form (Data Set 2) and the dried simplicia form (Data Set 1), as shown in Table IV.

These findings led to the application of the method to krangean leaves collected during an ethnopharmacological investigation of medicinal plants in Banjar Village, Licin District, Banyuwangi

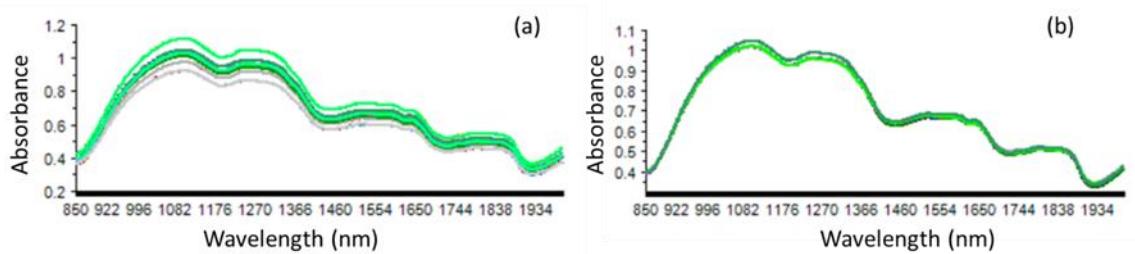


Figure 3. Visualization of the infrared spectra of the training set (a) and test set (b) of krangean leaf simplicia

Regency, with the goal of alleviating diarrhoea. A combination of herbal components, including quercetin flavonoid, was detected in krangean leaf simplicia collected from Banjar Village according to the findings obtained using chemometrics techniques.

Table IV. Evaluation of the dataset informed by the findings of chemometrics analysis using Linear Discriminant Analysis.

Data Set	LDA	
	% Recognition	% Prediction
1	90%	100%
2	96%	100%

DISCUSSION

This ethnopharmacy research was done to the subjects who are the Osing people of Banjar in Licin Subdistrict of Banyuwangi Regency by sending out questionnaires to the subjects which are included according to criteria. A significant Osing community in Banjar hamlet of Licin Subdistrict has maintained and continued some of their traditional ways of life (Singh et al., 2020). This was done in order to raise awareness over the traditional herbal medicine of diarrhoea. The choice of ethnopharmacological research according to Suryana and al. (2019) is based on the fact that ethnopharmacological research combines several methods to determine medicinal plants culturally significant as well as frequently used.

Ehnopharmacy (Laily et al., 2015) is not only on the cultural and social aspects about the use of medicinal plant, but also about the cure of diseases using medicinal plants. This method helps scientists better understand the intricate mesh of relationships that medicinal plants forage in the lives of people every day. The Osing people, however, regard krangean leaf as a main remedy for diarrhea and krangean leaf has been researched under ethnopharmacology. Some ethnopharmacology studies make use of

phytochemical screens and laboratory tests. In order to assess the chemical composition of krangean leaves, researchers implemented a phytochemical screening using the batteries of methods designed to separate different components. The findings of the phytochemical screening show that krangean leaves contain several bioactive chemicals maybe having therapeutic benefits for the community (Zaman et al., 2021). The flavonoid chemicals identified were sinaicitin, biochanin A, quercetin, chrysoeriol and astragalin. Flavonoids have also become famous for being antioxidant and anti-inflammatory and anticancer activities in the medical community (Thanh et al., 2023).

The next step is therefore to replicate krangean leaf conditions in order to perform chemometrics and Near Infrared (NIR) spectroscopy analyses. The compilation of ethnopharmacologically recognized therapeutic plants database: especially krangean leaves is the first step (Rinnan et al., 2009). The simulated krangean leaf samples are the krangean leaf herbal material blend with the flavonoid standard, pure krangean leaf, and quercetin flavonoid standard. Several biological uses for plants have been reported which contain a number of polyphenolic chemicals called flavonoids. Antioxidant and some other processes protect cells and tissues from harm by flavonoids, one of which is an antioxidant mechanism that neutralises the excess free radicals in the body. Additionally, cell membrane function is inhibited to change and decrease cell wall production by a mechanism that prevents microbial growth (Farag et al, 2020). Some research indicates that flavonoids may have the capacity to fight the bacteria and other microbial causes of the disease. Based on this, it is suggested that flavonoids may have therapeutic effects in the treatment of diarrhoea. This is consistent to the traditional medicine in Osing people of Banjar Village who treat diarrhoea by using powdered or dried krangean leaves (Laily et al., 2015). Definitely benefits and drawbacks of each of these

types have been clearly described. Whole or broken plant parts (dried simplicias) tend not to clump so well, they are easier to work with and store, but because simplicias must be ground or crushed before use, this might destroy volatile oils and other bioactive components. They are more prone to clumping because of the larger contact surface, however powdered simplicia (milled plant parts) offer better ease of accessibility for dose measurement and greater convenience for many uses like encapsulation or infusion in hot liquids.

Following that, a chemometrics classification model was created using infrared (IR) spectral analysis. This model was then used to krangean leaf specimens used by the Osing Tribe population in Banjar Village, Licin Subdistrict, Banyuwangi Regency. In order to find the right dataset model for classifying antidiarrheal compound content, this modelling effort was carried out to see if the model worked with specimens from the Osing tribe (Bevilacqua et al., 2017). Additionally, a Near Infrared (NIR) spectrophotometer was used to analyse the powdered specimens; there were twenty in the training set and five in the test set. Similarities in NIR spectral patterns might be due to comparable component compositions in the herbal material dataset that was analysed. On the other side, different herbal materials have different amounts of chemicals, which is why there are differences in absorbance. In most cases, the number of compounds is directly proportional to the absorbance value; in other words, a greater absorbance value indicates an increase in the number of compounds. Another factor that influences the correlation between absorbance values and component amounts is the wavelength employed to measure them. Higher absorbance levels are associated with longer wavelengths (Brereton et al., 2018). The data from the training set was then used to create a classification model.

For the purpose of this study, a multivariate calibration model was adopted. Results showed that the RMSEC (Root Mean Square Error of Calibration) was 0.0257 ($R^2_{\text{cal}} = 0.98$) and the RMSEP (Root Mean Square Error of Prediction) was 0.0277 ($R^2_{\text{val}} = 0.97$), both from the Partial Least Squares (PLS) model of dehydrated krangean leaf herbal material. The RMSEC and RMSEP values for the dehydrated krangean leaf powder herbal material were 2.87 ($R^2_{\text{cal}} = 0.9931$) and 0.0124 ($R^2_{\text{val}} = 0.9919$), respectively, according to the PLS model. In order to create the LDA model, we also took into account the RMSEC and RMSEP regression parameters from PLS. Whereas LDA

values reveal how well the LDA model can distinguish between clusters, RMSEC and RMSEP provide the average discrepancy between the predicted and actual values for calibration and prediction data, respectively. According to Amat-Ur-Rasool et al. (2020), LDA models provide more accurate predictions for calibration data when the RMSEC value is less than the RMSEP value, and for prediction data when the RMSEC value is more than the RMSEP value.

The spectral data retrieved from each of the datasets that comprised of the training set were used to create a chemometrics classification model known as Linear Discriminant Analysis (LDA). The LDA works better when the dataset is able to properly classify or differentiate among many classes and hence would be a better dataset as the accuracy of the dataset increases (Sima et al., 2021).

The structural forms of krangean leaf herbal material, including both the dried simplicia and powdered forms, were used to demarcate two separate datasets. The cross-validation approach may be used to test the accuracy and usefulness of the LDA. It can determine how effectively the LDA can forecast a group. However, the LDA's ability to detect unknown values may be determined by calculating its mean square error, which is a measure of the prediction efficacy (Kumar et al., 2021). In order to find the best model that shows a strong relationship between categories and discriminants, a thorough evaluation was carried out. The results show that there is a distinct difference between the categories of mixed and pure krangean leaf herbal materials, as shown by the mapping. Figures 1 and 2 show how the samples are categorised into the required groups in the prediction table.

The results of that table indicate that in powdered form, the Linear Discriminant Analysis (LDA) based on the infrared spectrum trained on training set 2 is the best chemometrics classifier of pure krangean leaf herbal material and mixed krangean leaf simplicia. Its recognition and prediction metrics are evaluated to 100% perfect score. For this reason, the krangean leaf samples obtained for the ethnopharmacy study should be transformed into a powdered form (Amat-Ur-Rasool et al., 2020). Within the framework of theoretical, this claim is consistent with the idea that dry powder simplicia offer improved efficiencies and precision of analytical processes by enhancing the contact between flavonoid chemicals and near infrared light.

Additionally, krangean leaf specimens were used in an ethnopharmacological study of medicinal plants for antidiarrheal purposes in

Banjar Village, Licin Subdistrict, Banyuwangi Regency. Table IV shows that chemometrics techniques were applied to krangean leaf simplicia samples collected near Banjar Village, Licin Subdistrict, Banyuwangi Regency. The results show that the samples are mixed simplicia, which means that they may contain quercetin flavonoid compounds.

CONCLUSION

The most common medicinal plant used by the Osing Tribe in Licin Subdistrict, Banyuwangi Regency, to cure diarrhoea, according to an ethnopharmacy research, is krangean leaf. However, other plants are also used for their ability to prevent diarrhoea. This is followed by the discovery of quercetin flavonoids in krangean leaves from Banjar Village, Licin Subdistrict, Banyuwangi Regency, which were analysed using Near Infrared and Chemometrics as antidiarrheal chemicals. More research on the antidiarrheal properties of medicinal plants utilised by the Osing Tribe in Banjar Village and other Licin villages should be undertaken.

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CONFLICT OF INTEREST

There is no personal interest that conflicts with this matter.

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