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Study on ethnopharmacy of medicinal plants for antidiarrheal purposes among the Osing Tribe using Near Infrared Spectroscopy and chemometrics techniques

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ABSTRACT: Traditional healing culture has long been known in Indonesia, this is proven by the existence of inscriptions that tell about herbal medicine that has been used for generations. As time goes by, the use of plants as medicine has decreased. This is because the inheritance process is only verbal and there is no documentation about these medicinal plants. This encourages research and preservation of plants as medicine, one of which is ethnopharmacy. The implementation of ethnopharmacy in the Osing tribe regarding the use of medicinal plants as anti-diarrhea can provide knowledge about the existence of plants that contain flavonoid compounds which have the potential to act as anti-diarrhea. This study aimed at verifying the anti-diarrheal attributes of medicinal plant compounds, *Near Infra-Red* (NIR) spectroscopy and chemometric techniques were employed. Leveraging the established principle of flavonoid compounds as potent antioxidants capable of stabilizing the digestive tract, we employed flavonoid standards as a reference benchmark for assessing the anti-diarrheal efficacy of these compounds. This approach facilitated a comprehensive evaluation to verify the extent of their anti-diarrheal potential. The results of this research found that the guava leaf plant is widely used by the Osing tribe as an anti-diarrhea. Next, guava leaves were analyzed using *Near Infra Red* (NIR) spectroscopy and chemometric techniques, and test results were obtained in the form of guava leaf samples from the Osing tribe that contain flavonoids so they can be used for antidiarrheal therapy.

KEYWORDS: Ethnopharmacy; antidiarrhea; spectroscopy; chemometrics.

1. INTRODUCTION

Within the Indonesian society, medicinal plants occupy a distinct position, representing a cultural heritage in the domain of health and standing out as a well-established cultural legacy renowned for its therapeutic efficacy. Consequently, these medicinal plants have been passed down through generations and frequently employed by the Indonesian population as integral elements of traditional medicine essential for the community [1]. The Osing region, situated around the Ijen Mountains and Banyuwangi Regency in East Java Province, exhibits a distinct cultural identity that encourages researchers seeking to explore this area [2]. While numerous publications delve into the religious rituals practiced by the Osing community, limited documentation exists regarding the essential knowledge of medicinal plant usage. This documentation serves as an important resource, providing pivotal insights and serving as a foundational exploration for the discovery of potential new medicines [3,4].

To explore the indigenous community's knowledge regarding the use of medicinal plants, an approach rooted in ethnopharmacy can be employed. Ethnopharmacy constitutes an interdisciplinary field focused on the utilization of medicinal plants within the context of a specific society's healing culture [5]. While extensive research exists on ethnopharmacy, particularly concerning various ethnic groups in Indonesia, a limited number of research has been directed towards the Osing community. Therefore, this study serves as an initial gateway to unveil the traditional medicinal practices of the Osing ethnic group in East Java, offering a vital starting point for further investigation [6].

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The detection of compound presence often involves employing analytical methodologies namely *Near Infra Red* (NIR) spectroscopy and chemometrics [7]. NIR spectroscopy operates on electromagnetic wavelengths beyond the visible light spectrum (specifically within the 700-3000 nm range), enabling for the identification of compounds in a given preparation or sample [8]. Its widespread application extends notably to the analysis of organic compounds. Additionally, chemometric techniques serve as a supporting approach for quantitative analysis, facilitating further exploration of the variations among constituent elements within a sample, thereby enabling precise measurement of compound quantities [9,10]. These techniques were employed to ensure validation and safety for consumers of medicinal plants to optimize the expected therapeutic outcomes [11].

Based on the aforementioned explanation, the researcher was encouraged to conduct an investigation focusing on the ethnopharmacology of anti-diarrheal medicinal plants within the Osing ethnic community. This study aims to employ *Near Infra Red* spectroscopy and chemometric techniques as instrumental tools in the research process.

2. RESULTS

Through ethnopharmacological investigations, it was observed that guava leaves are prominently used within the Osing ethnic group to manage diarrheal conditions. Subsequent phytochemical screening identified the presence of quercetin flavonoids in the extract [12]. The detailed outcomes are presented in Table 1.

Table 1. Phytochemical Screening of Guava Leaf Extract was conducted through several tests based on the suspected compound content within the extract using specific reagents.

Phytochemical Test	Reagent	Observation	Result
Alkaloid	• Extract + Mayer • Extract + Bouchardat • Extract + Mayer		+ + +
Flavonoid	• Extract + Mg powder + amyl alcohol • Extract + concentrated HCl		+ +
Tannin or Phenolic	• Extract + $FeCl_3$		+
Saponin	• Extract + hot water (shaken vertically for 10 minutes)		-
Triterpenoid or Steroid	• Extract + Liebermann Bourchat		+

Description:
+ : Contains secondary metabolite compounds
- : Does not contain secondary metabolite compounds

The next stage involves preparing simulated samples of guava leaf herbal material, followed by analysis using *Near Infra Red* (NIR) and chemometric techniques to establish a chemometric classification model. This model was then applied to guava leaf herbal material samples used by the Osing community in the village of Banjar, Licin District, Banyuwangi Regency.

A classification model was established using a training dataset. The spectra from each dataset were utilized to construct a chemometric classification model, specifically Linear Discriminant Analysis (LDA) [13]. From the results of the infrared scan in Figure 1, the entire infrared spectra generated from both pure guava leaf herbal material and the mixture in the training set and test set showed similar absorption patterns, differing only in the quantitative values of their respective spectrum absorbances. The outcomes demonstrated a mapping illustrating the differentiation between pure guava leaf herbal material and mixtures. Meanwhile, the prediction table exhibited the classification of samples into the established categories.

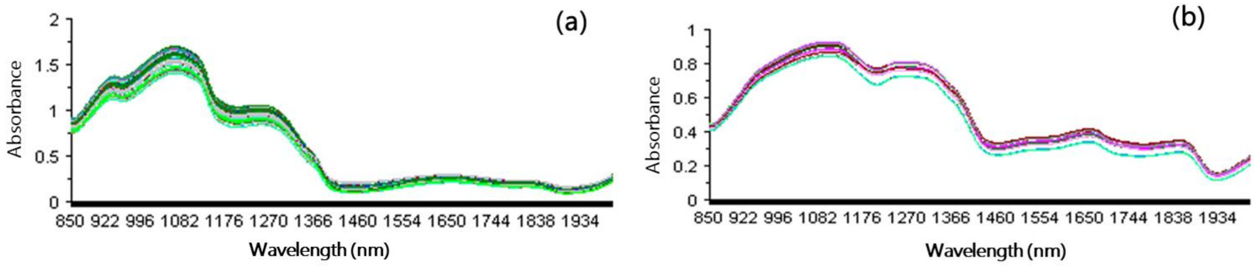
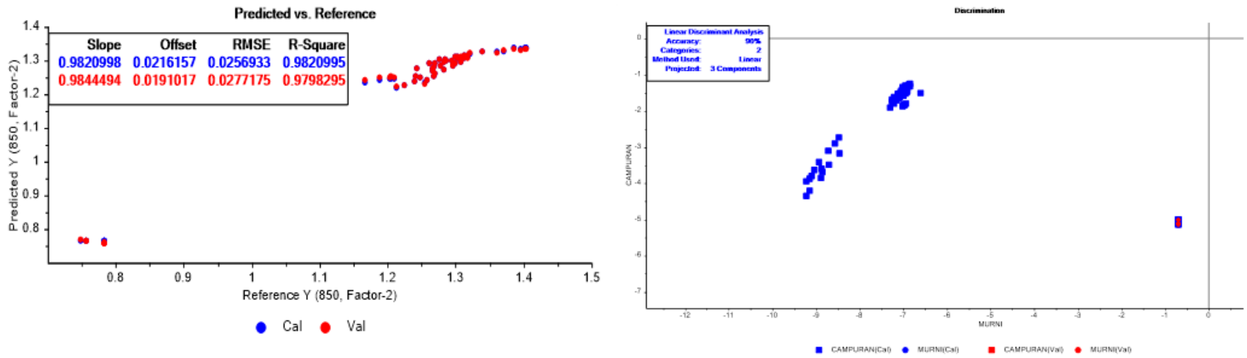


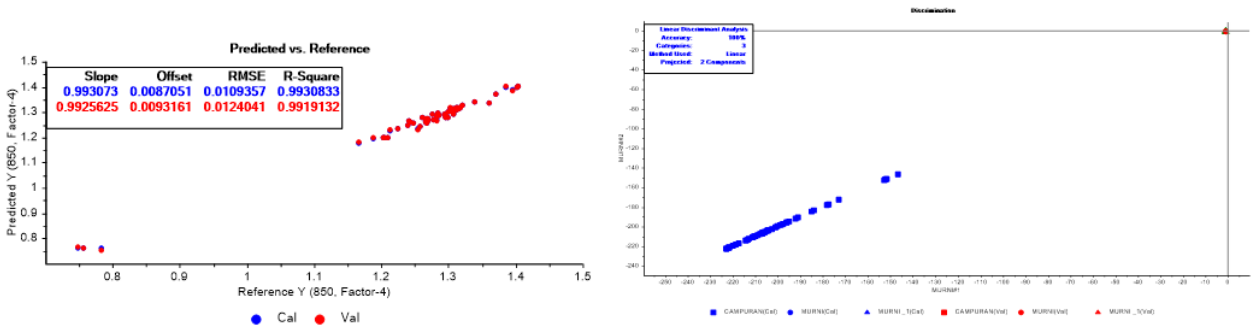
Figure 1. Visualization of the infrared spectra of the training set (a) and test set (b) of guava leaf simplicia

Subsequently, the establishment of a classification model was undertaken utilizing the training set data. The spectra derived from each dataset within the training set were employed to formulate a chemometric classification model, specifically employing Linear Discriminant Analysis (LDA). Consequently, a systematic evaluation was conducted to discern the most optimal model that establishes a meaningful association between categories and discriminants. The results, in the form of mapping, demonstrate a distinct segregation between the categories of pure and mixed guava leaf herbal material on dried leaf (Figure 2) and dried powder leaf of guava (Figure 3).



(a) (b)

Figure 2. The model properties of the dried guava leaf herbal material training set in (a) partial least square (PLS) with R-Square values and (b) Linear Discriminant Analysis (LDA) with accuracy values.



(a) (b)

Figure 3. The model properties of the dried guava leaf powder herbal material training set in (a) partial least square (PLS) with R-Square values and (b) Linear Discriminant Analysis (LDA) with accuracy values.

Based on the data of the Linear Discriminant Analysis, the %recognition and %prediction values of each model formed based on the shape of the consumed guava leaves are obtained, namely the dried simplicia form (Data Set 1) and the powder form (Data Set 2) as shown in Table 2.

Table 2. Assessment of the dataset based on the results of chemometric analysis in Linear Discriminant Analysis.

Data Set	LDA	
	% Recognition	% Prediction
1	80%	90%
2	100%	100%

Subsequently, an application was conducted on guava leaf samples obtained from the ethnopharmacological study of medicinal plants for anti-diarrheal purposes in the Village of Banjar, Licin District, Banyuwangi Regency. Based on the results obtained using chemometric techniques applied to guava leaf simplicia collected from the area of Banjar Village, Licin District, Banyuwangi Regency, the samples indicated a mixed herbal material showing the presence of quercetin flavonoids.

3. DISCUSSION

This ethnopharmacy research was conducted among the Osing tribe in the village of Banjar, Licin Subdistrict, Banyuwangi Regency, utilizing a questionnaire distributed to respondents who met the inclusion criteria. The selection of this location was based on the substantial Osing population residing in Banjar village, Licin Subdistrict, where the majority still adheres to cultural practices passed down through generations [14]. This choice aimed to deepen information concerning the traditional treatment of diarrhea using medicinal plants. The ethnopharmacy study was chosen because it includes several methods for determining which medical plants are widely used and their properties in society. Ethnopharmacy does not only focus on medicinal plants themselves, but also on the cultural and social aspects associated with their use. This allows researchers to understand in more depth how medicinal plants are integrated into people's live. The ethnopharmacy results revealed that guava leaf is the most frequently consumed plant by the Osing tribe to cure diarrhea [15,16]. Subsequently, the methods used in ethnopharmacy studies include laboratory analysis or phytochemical screening. Phytochemical screening was performed to investigate the compound composition present in guava leaves using various methods tailored to identify specific compounds. The phytochemical screening results indicated that guava leaves contain several bioactive compounds with potential therapeutic benefits for the community [17].

The next stage involves the preparation of simulated guava leaf samples followed by analysis using Near Infrared (NIR) spectroscopy and chemometrics. This preparation entails creating a dataset using medicinal plants identified through ethnopharmacy, specifically guava leaves [18]. The simulated guava leaf samples include pure guava leaf, quercetin flavonoid standard, and a mixture of guava leaf herbal material with the flavonoid standard. Flavonoids have been shown to have potential benefits in treating diarrhea with potential mechanisms as antibacterial activity so potentially helping fight off diarrhea-causing bacteria. This aligns with the practices of the Osing tribe in Banjar Village, who commonly consume guava leaves in the form of dried or powdered leaves to heal diarrhea [16]. Both forms have advantages and disadvantages.

Dried simplicias (whole or chopped plant parts) are easier to handle and store without clumping, but it requires grinding or crushing before use, which can lead to loss of volatile oils and other active components. Whereas powder simplicia (ground plant parts) are easier to measure doses and more convenient for more applications (e.g., capsules or hot drink) but they have higher risk of clumping due to increased surface area contact.

Afterwards, the IR spectrum was utilized to form a chemometric classification model and its application to the guava leaf samples used by the Osing Tribe community in Banjar Village, Licin Subdistrict, Banyuwangi Regency. This modeling was conducted to ensure which model was appropriate for application to samples found in the Osing tribe community, thereby determining the proper dataset model for the classification of anti-diarrheal compound content [19]. The powder samples, totaling twenty for the training set and five for the test set, were also measured using a Near Infrared (NIR) spectrophotometer. The similarity in NIR spectra patterns may arise due to the presence of similar compound contents in the tested herbal material dataset. Meanwhile, variations in absorbance can be attributed to differing quantities of compounds in each tested herbal material [20].

Subsequently, the establishment of a classification model was undertaken utilizing the training set data. The spectra derived from each dataset within the training set were employed to formulate a chemometric classification model, specifically employing Linear Discriminant Analysis (LDA). LDA hinges on the dataset's capacity to effectively categorize or discriminate one class from another, with a higher degree of precision indicative of superior dataset performance [21,22]. Two distinct datasets were delineated based on the structural forms of guava leaf herbal material, encompassing both the dried simplicia and powdered forms [23]. Consequently, a systematic evaluation was conducted to discern the most optimal model that establishes a meaningful association between categories and discriminants. The results, in the form of mapping, demonstrate a distinct segregation between the categories of pure and mixed guava leaf herbal material. Meanwhile, the prediction table presents the classification of samples into the designated categories, as depicted in Figures 2 and 3.

Subsequently, testing was conducted to assess the model's ability to distinguish between the two types of guava leaf herbal material, as evident in the recognition capability values for samples in the training set and the predictive ability of LDA for samples in the test set. It was found that the LDA model of dried guava leaf herbal material resulted RMSEC and RMSEP values of 0.0257 (R^2_{cal} = 0.9821) and 0.0277 (R^2_{val} = 0.9798), respectively. Whereas, the LDA model of dried guava leaf powder herbal material resulted RMSEC and RMSEP values of 0.0109 (R^2_{cal} = 0.9931) and 0.0124 (R^2_{val} = 0.9919), respectively. Table 2 demonstrates that the optimal chemometric classification model for pure guava leaf herbal material and mixed guava leaf simplicia is the LDA model based on the IR spectrum in training set 2, in powder form, where the recognition and prediction values are both 100%. Hence, the guava leaf samples obtained from the ethnopharmacy study need to be processed into dry powder form [24]. This is inline with the theory that dry powder simplicia have a form that easier for interaction of flavonoid compounds with *near infra red*, so that the results of the analysis process can be identified more quickly and accurately.

Next, an application was carried out on guava leaf samples obtained from the ethnopharmacy study of medicinal plants for antidiarrheal purposes in Banjar Village, Licin Subdistrict, Banyuwangi Regency. Based on the results in Table 2, utilizing the chemometric techniques conducted on guava leaf simplicia collected in the area of Banjar Village, Licin Subdistrict, Banyuwangi Regency, it indicates that the samples are categorized as mixed simplicia, demonstrating the presence of quercetin flavonoid compound content.

4. CONCLUSION

From the results of the ethnopharmacy study on medicinal plants for anti-diarrheal purposes, the most widely consumed by the Osing Tribe in Licin Subdistrict, Banyuwangi Regency, is guava leaf. However, there are other plants also used as anti-diarrheals. Subsequently, the identification of anti-diarrheal compounds in guava leaves in Banjar Village, Licin Subdistrict, Banyuwangi Regency, using Near Infrared and Chemometrics indicates the presence of quercetin flavonoid content. A recommendation for further research is to conduct anti-diarrheal activity tests on medicinal plants used for this purpose by the Osing Tribe in Banjar Village or other villages within the Licin Subdistrict of Banyuwangi Regency.

5. MATERIALS AND METHODS

5.1. Material

The materials in this study utilized samples of medicinal plants (comprising dried herbal material and reference compounds); quercetin flavonoid standard with ChromaDex analysis certificate from PT. Dexa Medica; and samples of medicinal plants from the Osing Tribe.

5.2. Tools

A questionnaire attached with ethical approval was used the survey stage. The instruments used in this study were analytical balance, oven, blender, sieve with size B-30. A set of near-infrared spectrometer (Brimrose Corporation Luminar 3070) was exploited for data acquisition process coupled with BRIMROSE software and The Unscrambler X 10.2 software for data analysis.

5.3. Ethnopharmacy study

This research commenced with a preliminary survey, followed by data collection through an ethnopharmacy approach conducted in the Licin Subdistrict, Banyuwangi Regency. The selection of the village was based on the predominant presence of the Osing Tribe in the community. Subsequently, chemical content testing on the collected samples will be carried out in the Chemistry Laboratory of the Faculty of Pharmacy at the University of Jember.

The ethnopharmacy study took place in Banjar Village, Licin Subdistrict, Banyuwangi Regency (Latitude: -8.193077160373061; Longitude: 114.26683913916348; R748+QPM). Ethical approval for the ethnopharmacy study was obtained, submitted to Universitas dr. Soebandi Jember, with the reference number: 441/KEPK/UDS/IX/2023. Following this, data collection was conducted using interview and questionnaire methods involving 96 samples from the Osing Tribe in Banjar Village. The ethnopharmacy results identified guava leaf as a frequently used plant to address diarrhea-related complaints among the Osing Tribe in Banjar Village, Licin Subdistrict, Banyuwangi Regency. The guava leaf samples were then determined at the Herbal Materia Medica Laboratory in Batu, with herbarium number: 000.9.3/2911/102.20/2023.

5.4. Sampel preparation

The sample preparation was carried out by combining simplicia and compounds. The prepared simulated samples consisted of plant simplicia and compound mixtures. The simulated samples were divided into two groups, namely the training set and test set. Guava leaf simplicia samples mixed with quercetin flavonoid were prepared at various concentrations within the quercetin flavonoid concentration range of 1-100% of the total (± 10 milligrams), as presented in Table 3.

5.5. Chemometrics techniques

The sample to be measured was placed on the sample holder located in the Integrating Sphere unit. Light from the halogen lamp would pass through several sets of equipment and be filtered as specified. After the light hits the sample, the reflection of near-infrared light would be captured by the sensor and undergo digitization. Measurements on the NIR Spectrophotometer employed a filter with a wavelength range of 1400-2000 nm and a data collection interval of 5 nm, resulting in obtaining reflection data for a total of 120 points.

In chemometric analysis using The Unscrambler X 10.2 software, the selection of the classification model was based on the best recognition ability and prediction ability. Recognition ability is defined as the percentage of correct classification by the model against the training set samples, while prediction ability is defined as the percentage of correct classification by the model against the test set samples. Quality of the chemometrics models were assessed according to the values of the calibration determination coefficient (R^2_{cal}), validation determination coefficient (R^2_{val}), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).

Table 3. The Training Set data was created with a composition of guava leaf simplicia and quercetin flavonoid.

Quercetin Flavonoid (gram)	Guava Leave Herbal Material (gram)	Concentration (%)	Category
0	10	0%	Pure
0.011	10	1.1%	Mixture
0.021	10	2.1%	Mixture
0.032	10	3.2%	Mixture
0.040	10	4%	Mixture
0.051	10	5.1%	Mixture
0.060	10	6%	Mixture
0.073	10	7.3%	Mixture
0.082	10	8.2%	Mixture
0.092	10	9.2%	Mixture
0.1015	10	10.05%	Mixture
0.2040	10	20.14%	Mixture
0.3028	10	30.18%	Mixture
0.4004	10	40.03%	Mixture
0.5014	10	50.10%	Mixture
0.6005	10	60.00%	Mixture
0.7038	10	70.00%	Mixture
0.8022	10	80.01%	Mixture
0.9033	10	90.93%	Mixture
1.0011	0	100.00%	Mixture

The test set consists of five sets of simulated guava leaf herbal material data, namely pure guava leaf raw material, quercetin flavonoid material, and guava leaf raw material mixture with concentrations of 20%, 50%, and 70%, as shown in Table 4. Pure guava leaf herbal material is categorized within the mixed guava leaf herbal material to evaluate the formed model.

Table 4. Test set data with a composition of guava leaf herbal material and quercetin flavonoid.

Quercetin Flavonoid (gram)	Guava Leave Herbal material (gram)	Concentration (%)	Category
0	10	0%	Pure
0.2040	10	20.00%	Mixture
0.5014	10	50.05%	Mixture
0.703	10	70%	Mixture
1.0011	0	100%	Mixture

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