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Rapid Detection and Quantification of Gambir Adulteration Using ATR-FTIR Spectroscopy Coupled with Chemometric Analysis

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Abstract

Gambir (an extract from *Uncaria gambir* Roxb.) is a valuable Indonesian herbal product susceptible to adulteration for economic gain. In this study, we developed a rapid, non-destructive method to detect and quantify adulteration in gambir using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy combined with chemometric analysis. In total, 32 gambir samples were prepared, including authentic gambir, samples adulterated with a fertilizer (SP36) at 0–50% w/w, quality control (pooled) samples, and a market sample of unknown purity. FTIR spectra (4000–600 cm^{-1}) were collected. The spectral data were preprocessed and analyzed with PCA, SIMCA, and PLS-R. PCA revealed clear clustering of samples according to adulterant concentration, with the first two principal components capturing more than 99% of total variance. Chemometric classification models successfully distinguished authentic gambir from adulterated samples: SIMCA showed clear separation, with the 1% adulterant concentration sample clustering near authentic gambir, while the PLS-R calibration model achieved excellent linearity ($R^2 \approx 0.988$) in predicting adulterant levels, with low RMSECV (≈ 0.0375) and a detection limit of 5.78% adulterant concentration. When applied to a gambir sample obtained from the market, the PLS-R model indicated ~25% adulterant concentration. These results demonstrate that FTIR spectroscopy combined with multivariate analysis can effectively detect and quantify even low levels of adulterant concentration in gambir. This approach offers a fast and reliable tool for quality control and authentication of herbal products prone to adulteration.

Keywords: *Gambir, Adulteration, FTIR, Chemometrics, Herbal Product Authentication.*

Introduction

Gambir is a dried extract from the leaves and twigs of the gambir plant (*Uncaria gambir* Roxb.) that serves as an important commodity in Indonesia[1],[2]. Its high market value and an annual export volume of thousands of tons have led to unscrupulous practices where some producers adulterate gambir by adding foreign substances to increase weight and profit[3],[4]. Adulteration is generally defined as the practice of partially or wholly replacing an authentic material with other substances of similar appearance for economic gain[5]. Such practices deceive consumers and can compromise the efficacy and safety of herbal products[6]. In the case of gambir, quality may also be reduced by poor processing methods. Reports suggest that materials such as soil, sand, starch, or even fertilizers have been used as adulterants in

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gambir, resulting in inferior product quality[7]. These adulterants are often fine powders that resemble gambir in color and texture, allowing them to be blended into gambir without easy visual detection. Because gambir is sold in a dried, block or powdery form, it can be particularly susceptible to this kind of chemical adulteration – inexpensive inorganic or organic fillers can be mixed in to increase bulk, with minimal changes in appearance or odor to alert buyers. These issues highlight the need for a reliable method to detect gambir adulteration and ensure product authenticity.

Vibrational spectroscopy, especially infrared (IR) spectroscopy, has emerged as a powerful tool for quality control of foods and herbal materials[8]. Infrared spectroscopy offers rapid and non-destructive analysis for identifying chemical composition and has been successfully used to evaluate the quality and authenticity of various functional and herbal products[9],[10]. However, IR spectral data are complex, and interpreting them directly can be challenging due to overlapping peaks and baseline variations[11]. To address this, multivariate statistical techniques (chemometrics) are applied to IR data to extract meaningful information and build predictive models[11],[12]. Chemometric analysis can filter out noise, reveal hidden patterns, enhance interpretability of spectra, and provide a visual approach to data analysis[11]. In other words, combining IR spectroscopy with chemometrics allows both qualitative and quantitative analysis of complex mixtures by leveraging the full spectral fingerprint of a sample[13]. This approach has been widely used for authentication and adulteration detection in foods and herbal medicines[13]. For example, near- and midinfrared spectroscopy coupled with chemometrics has been applied to discriminate herbal products and detect adulterants[10],[13]. Specific studies have demonstrated the effectiveness of IR fingerprinting to identify adulteration in spices like black pepper[14], and to authenticate botanical extracts and ingredients[13]. FTIR-based methods have also been employed to detect adulteration in other natural products – for instance, to screen for sugar syrup adulterants in honey[15],[16] – and to ensure the purity of essential oils[13]. In the domain of herbal medicine, IR spectroscopy with chemometric modeling has been used to confirm the authenticity of red ginger and detect its oil adulteration[17], as well as to analyze phytochemical contents in medicinal plant extracts[18]. These examples illustrate the broad applicability of spectroscopic fingerprinting combined with chemometrics for quality assurance in natural products[8],[9],[12],[13].

Despite the growing use of spectroscopy and chemometrics for detecting food and herbal adulteration, no published research has specifically addressed adulteration detection in gambir. Given gambir's economic importance and the adulteration issues observed in practice, there is a clear need for a method to authenticate gambir. Therefore, in this study we employ Fourier Transform Infrared (FTIR) spectroscopy combined with several chemometric techniques to detect adulterants in gambir. We focus on developing classification models to distinguish authentic gambir from adulterated gambir, and a calibration model to predict the percentage of adulterant present. By doing so, we aim to provide a scientific basis for rapid, reliable gambir authenticity testing and to highlight the broader contribution of spectroscopic chemometrics in combating herbal adulteration.

Experimental

Material

Authentic gambir was obtained in solid lump form from Pesisir Selatan, West Sumatra, Indonesia. The adulterant used in this study was SP36, a phosphate fertilizer. SP36 was selected as a representative foreign substance that unscrupulous suppliers might mix into gambir because it is a widely available fertilizer (containing ~36% P_2O_5) with a powdery form and pale color, making it easy to blend with gambir without noticeably altering the appearance. Prior to adulteration experiments, the gambir lumps were dried and ground into a fine powder using a mortar and pestle, then sieved through a 100-mesh sieve to ensure

uniform particle size. To verify the quality and purity of the base material, the moisture content and ash content of the gambir powder were measured according to standard procedures. The authentic gambir was found to have a moisture content of 13.90% and ash content of 3.83%, which meets the Indonesian national standard (SNI) for first-grade gambir (maximum 14% moisture and 5% ash), confirming that the gambir sample was of high purity (i.e. free of extraneous inorganic matter).

Methods

Adulterated Samples

Adulterated gambir samples were prepared by deliberately mixing the gambir powder with the SP36 adulterant at various percentage levels. Two groups of samples were prepared: a training set for model development and a test set for model validation. For the training set, eight different adulteration levels were created (0%, 1%, 2%, 5%, 10%, 15%, 25%, and 50% adulterant concentration). A 0% adulterant concentration (authentic gambir) served as the authentic reference, while the other levels simulated increasing degrees of contamination. Each formulation was prepared in triplicate to account for experimental variability, yielding 24 training samples in total. To prepare a given adulterated sample, the appropriate proportions of gambir powder and SP36 powder (for example, 990 mg gambir + 10 mg adulterant for a 1% adulterant concentration) were carefully weighed and thoroughly homogenized. All training set samples were then divided into two portions: the majority of each sample (~950–1000 mg) was reserved for spectral analysis, and a small aliquot (50 mg from each sample) was pooled with others to create quality control samples as described below.

Quality Control (QC) Samples

In chemometric analysis, quality control samples are often used to monitor the stability and reproducibility of measurements[19]. A common practice is to prepare a composite QC sample by mixing equal small portions from each of the study samples, creating a pooled sample that effectively represents the average composition (or adulteration level) of all samples[20]. In this study, a composite QC sample was prepared by taking equal small portions (50 mg) from each of the 24 training samples and mixing them together thoroughly. This pooled sample represents an average of all adulteration levels. The composite was then split into three aliquots, yielding three QC samples to be run at intervals. The purpose of these QC samples was to check the consistency of the FTIR instrument's response and the sample preparation throughout the experiment[21]. In PCA score plots, properly prepared QC samples are expected to cluster tightly, ideally near the center of the sample distribution, indicating good analytical precision[19].

Test Set

For external validation of the models, a test set of five samples was prepared. Four of these were adulterated gambir samples at intermediate concentrations that were not all included in the training set: 0% (authentic gambir), 1%, 15%, and 50% adulterant concentration (each prepared independently from the training samples). These serve as blind samples to test classification and prediction performance. Additionally, one market gambir sample (purchased from a local supplier in the Lima Puluh Kota region) was included as an unknown to be analyzed. The authentic purity of this market sample was not known a priori; it was treated as an independent sample to demonstrate the real-world applicability of the method.

FTIR Spectral Acquisition

All samples (training, QC, and test) were analyzed using Perkin Elmer Spectrum 100 Fourier Transform Infrared spectroscopy in Attenuated Total Reflectance mode (ATR-FTIR). Approximately 1 gram of each sample (or the maximum amount sufficient to cover the ATR crystal) was placed on the ATR crystal (a ZnSe crystal element) of a PerkinElmer FTIR spectrometer. The spectrum for each sample was collected over the mid-infrared range of 4000–

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600 cm^{-1} with appropriate instrument settings (each measurement consisted of multiple scans coadded to improve signal-to-noise ratio, at a resolution of 1 cm^{-1}). The ATR technique provides direct measurement of solid powder samples without further preparation and yields an IR absorbance (or transmittance) spectrum characteristic of the sample's chemical constituents. Background spectra (with no sample) were recorded periodically and subtracted from sample spectra automatically by the instrument's software. The FTIR spectra for all 32 samples were thus obtained, producing absorbance values at each wavenumber (frequency) point. These spectral data were exported for chemometric analysis as a matrix of absorbance intensity (X variables) vs. wavenumber.

Data Preprocessing

Prior to building chemometric models, the spectral data were preprocessed to remove unwanted variations and enhance relevant features[22],[23]. First, a min–max normalization was applied to each spectrum. This linear scaling normalized the absorbance values so that each spectrum had a comparable range (often scaling the values between 0 and 1)[22]. Such normalization helps correct for differences in overall intensity that can arise from slight variations in sample amount or ATR contact pressure. Next, Multiplicative Scatter Correction (MSC) was used as a filtering step to eliminate multiplicative scattering effects and baseline shifts[24]. MSC adjusts each spectrum by estimating and removing the influence of scattering, effectively aligning all spectra to a common baseline and scale. This preprocessing is essential to improve the accuracy of subsequent multivariate analysis by focusing the data on chemical variations rather than physical or instrumental variations[22],[24].

Chemometric Analysis

All chemometric analyses were performed using SIMCA v.14.1 software (Umetrics, Umeå, Sweden). The workflow encompassed unsupervised exploration, supervised classification, and regression modeling. The results were visualized in terms of score plots, loading plots, and prediction plots as needed. Key outcomes from these analyses are described in the Results and Discussion section.

Principal Component Analysis (PCA)

PCA was first applied to the processed spectral dataset to explore natural groupings and detect any outliers[25]. PCA reduces the high-dimensional spectral data into a few principal components (PCs) that capture the majority of variance in the dataset[25]. By examining PCA score plots (plots of samples in the coordinate space of the first few PCs), we could visualize clustering of samples according to adulteration levels[15]. PCA also allowed us to check the QC samples' clustering as an indicator of analytical precision[20]. In our analysis, a scatter plot of the first principal component (t1) vs. the second (t2) was used as an overview of the data structure[25]. The percentage of total variance explained by these PCs was noted to assess how well the data compression preserved the information (typically, >70% variance by two PCs is considered a good representation in chemical data sets)[26].

Soft Independent Modeling of Class Analogy (SIMCA)

SIMCA was used to build a classification model for gambir authenticity[25],[27]. In SIMCA, separate PCA models are created for each predefined class (in this case, classes could be defined as "authentic gambir" vs. various "adulterated gambir" classes)[25],[28]. Each class model retains enough principal components to describe most variance of that class, determined by internal cross-validation[28]. New samples are then classified by their distance to each class model in multivariate space[28]. We developed SIMCA models for our data, and utilized Coomans plots to evaluate class separation. A Coomans plot is a diagnostic that plots the distance of samples to two class models simultaneously, aiding in visualization of how well classes (e.g., authentic vs. adulterated) are separated[28]. A statistical distance threshold

(D_{Crit}) at 95% confidence was used to delineate class membership on these plots (corresponding to Hotelling's T^2 ellipse at 95% in PCA space). In essence, SIMCA provided an independent modeling approach to confirm whether authentic gambir samples form a distinct cluster apart from adulterated ones.

Partial Least Squares Regression (PLS-R)

To quantify the amount of adulterant in gambir, we developed a PLS-R model using the training set spectra (X matrix) and the known adulterant concentrations (Y vector)[28],[29]. PLS-R is particularly suited for such data where spectral variables are numerous and collinear. It finds latent factors that correlate with the adulterant concentration, effectively building a calibration equation to predict adulterant concentration (%) from a sample's IR spectrum[30]. The PLS-R model was optimized by selecting the appropriate number of latent variables (factors) via cross-validation to avoid overfitting[30]. We evaluated the quality of the PLS-R model by the calibration R^2 (how well the model fits the training data), and by the root mean square error of calibration (RMSEC) and cross-validation (RMSECV) – lower values of these indicate better predictive accuracy[31]. Once the model was finalized, it was used to predict adulteration levels for the test set samples, and the predictions were compared against true values for validation[31]. We also calculated the Limit of Detection (LOD) and Limit of Quantification (LOQ) for adulterant concentration from the regression model according to ICH Q2(R1) using the standard deviation of a linear response and a slope[32]. LOD was defined as the lowest adulterant concentration that could be statistically distinguished from 0% within a given confidence (generally taken as 3.3× the standard deviation of prediction error), and LOQ as the lowest concentration that could be quantified with acceptable accuracy (often 10× the standard deviation of prediction error)[32]. In practice, we used the residual standard deviation of the PLS calibration (Sy/x) and the calibration slope to estimate these values, following standard guidelines: approximately, LOD ≈ 3.3*(Sy/x)/slope and LOQ ≈ 10*(Sy/x)/slope[29]. This approach is equivalent to the 3σ and 10σ criteria mentioned above, and provides an instrument- and model-based estimate of the method's sensitivity for detecting low-level adulteration.

Results and Discussion

IR Spectral Characteristics and Data Pretreatment

The ATR-FTIR spectra of authentic and adulterated gambir samples showed complex profiles characteristic of organic plant material mixed with inorganic adulterant (Figure 1a). Gambir extract contains compounds such as catechins, flavonoids, and other polyphenols, which contribute IR absorption bands (for example, O–H and C=O stretches in the region ~3200–2400 cm^{-1} and ~1700 cm^{-1}, respectively, and C–O vibrations in the fingerprint region). The adulterant (SP36 fertilizer, primarily calcium phosphate and related minerals) contributes different absorption features, particularly in the fingerprint region (phosphate vibrational bands often appear around 1100–900 cm^{-1}). In the raw spectra of the samples, as adulterant concentration increased, subtle changes in certain peak intensities and shapes were observed (such as increasing prominence of phosphate-associated bands and attenuation of some organic bands). However, baseline shifts and scatter effects due to sample preparation could obscure these differences in the unprocessed data.

Infrared absorption bands correspond to specific molecular vibrational modes – primarily **stretching** (bond length vibrations) and **bending** (bond angle vibrations). In organic constituents of gambir, broad O–H stretching bands (due to hydroxyl groups in polyphenols and residual moisture) appear around 3600–3000 cm^{-1}, while C–H stretching modes of aliphatic groups are seen near 2900 cm^{-1}. Strong C=O stretching vibrations of carbonyl groups (e.g., in catechins) give absorbance peaks around 1650–1550 cm^{-1}, and medium aromatic C=C stretches appear in the 1550–1500 cm^{-1} region.

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Table 1. Key IR bands observed in authentic vs. adulterated gambir samples (ATR-FTIR).

Wavenumber (cm^{-1})	Vibrational Assignment	Authentic Gambir (Black)	Change with Adulteration (Red, SP36)
3210 (broad)	O–H stretching (polyphenols, moisture)	Strong broad band	Relative intensity decreases; baseline shift more evident after adulteration
2932	C–H stretching (aliphatic CH_2, CH_3)	Medium intensity peaks	Slight decrease in relative intensity with higher adulterant
1615	C=O stretching (carbonyl in catechins/flavonoids)	Distinct sharp peak	Decreased intensity in adulterated samples
1520	Aromatic C=C stretching (phenolic/aromatic rings)	Present	Peak diminishes with adulteration
~1100–1000	P–O stretching (phosphate from SP36)	Absent/weak	Strong emerging peaks in adulterated samples, intensity increases with adulterant level
~600	O–P–O bending (phosphate)	Absent	Clear band visible in adulterated samples

In the fingerprint region (1500–600 cm^{-1}), various C–O stretching and O–H bending modes from gambir's organic compounds are present. In contrast, the inorganic phosphate in SP36 exhibits characteristic P=O stretching bands roughly in the 1100–1000 cm^{-1} range, and O–P–O bending vibrations at lower wavenumbers near ~600 cm^{-1}. Understanding these vibrational assignments helps interpret how the spectra change with adulteration (**Table 1**).

After applying min–max normalization and MSC preprocessing, the IR spectral data were notably improved for analysis (Figure 1b). The MSC-corrected spectra had nearly identical baselines and reduced scattering differences, making the relative intensity changes more attributable to compositional differences. For instance, after correction, samples with higher adulterant concentration clearly showed greater relative intensity in the 1100–1000 cm^{-1} region (attributed to phosphate) compared to authentic gambir. Meanwhile, the broad O–H stretch region (around 3300 cm^{-1}) and C–H stretching region (~2900 cm^{-1}) were more consistent across samples after normalization, indicating successful mitigation of pathlength variability. These preprocessing steps ensured that subsequent multivariate modeling would highlight genuine chemical variation (due to adulterant concentration) rather than artifacts.

PCA Exploratory Analysis

Principal Component Analysis of the preprocessed spectra provided an initial overview of the data structure. PC1 and PC2, explained approximately 68.8% and 22.2% of the total variance, respectively, giving a cumulative total of over 91% (Figure 2). This high cumulative variance indicates that the majority of information in the IR spectra (relevant to differences among samples) can be captured in a two-dimensional scores plot. In the PCA score plot (PC1 vs. PC2), clear clustering according to

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adulteration level was observed. Authentic gambir samples (0%), along with low-level adulterated samples (1% and 2%), clustered closely in the lower region of the plot (i.e., lower t[1] values), suggesting strong spectral similarity and minimal chemical deviation. In contrast, samples with higher adulterant concentration (10% to 50%) progressively shifted to the left along PC2, forming a distinct vertical gradient. This gradient indicates that PC2 effectively captured the adulteration trend, with increasing adulterant concentration driving greater deviation from authentic spectral profiles.

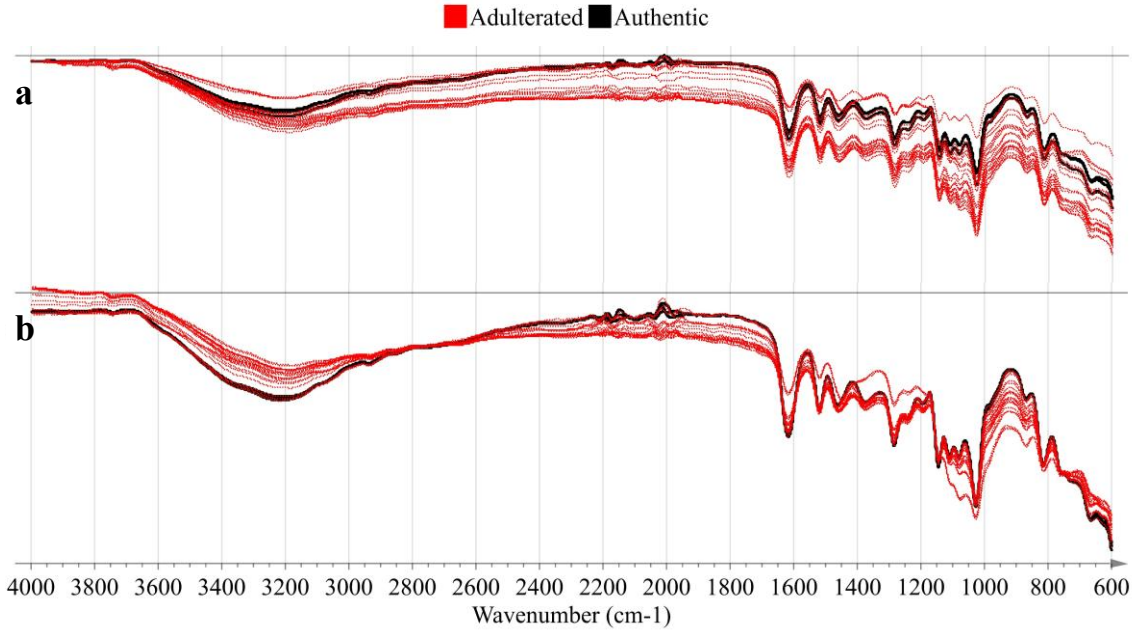

Figure 1. FTIR–ATR absorbance spectra of authentic gambir and adulterated gambir (with SP36) across the mid-infrared region (4000–600 cm^{-1}): a) raw spectra (no preprocessing), and b) after normalization and MSC preprocessing.

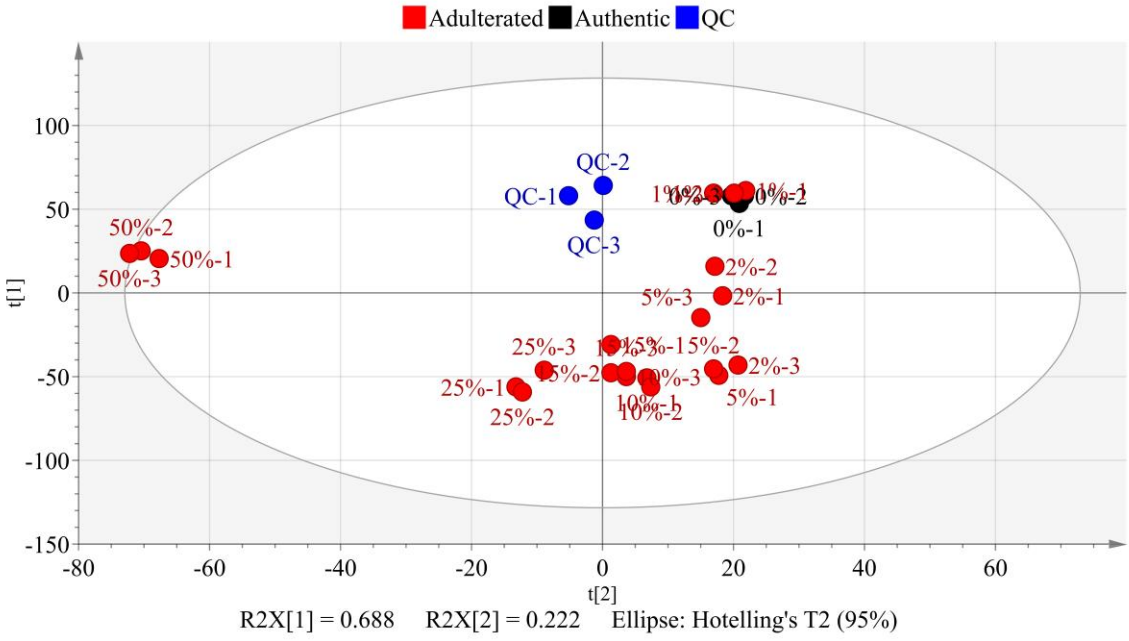

Figure 2. PCA score plot based on the preprocessed FTIR–ATR spectra, showing samples of authentic gambir (black points) and adulterated gambir (red points) plotted on the first two principal components (PC1 vs. PC2). Blue points denote QC samples.

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The quality control (QC) samples (blue points) appeared on the PCA plot as well (Figure 2). It also clustered tightly near the authentic region, specifically adjacent to the 0% and 1% samples. Ideally, the three QC replicates (being identical mixtures) should overlap or form a tight cluster. In our results, the QC samples did cluster together, but they were not exactly in the center of the overall sample distribution as one might expect. They were slightly offset, which we suspect was due to minor cumulative weighing errors when preparing the pooled QC mixture. Despite this, the proximity of QC points to each other indicates that the instrument and method were stable throughout the analysis.

Overall, the PCA plot revealed a coherent structure in the data, with adulteration levels forming an ordered pattern along PC1, and no major outliers observed. The dominant influence of a single factor (adulterant concentration) driving spectral variance supports the potential for building robust classification and quantification models. Notably, a two-component PCA model capturing more than 91% of the variance far exceeds the 70% threshold typically cited for interpretability in chemometric studies, underscoring the strong discrimination power of the spectral data.

SIMCA Classification of Authentic vs. Adulterated Gambir

To classify gambir samples and detect adulteration qualitatively, a SIMCA model was built using the PCA sub-models of each class. For simplicity, we defined two broad classes Authentic Gambir (0% adulterant concentration) and Adulterated Gambir (containing any amount of adulterant concentration). The training data (with known classes) were used to develop a PCA model for each class. The authentic gambir class naturally had very little internal variation (being just one material), and the adulterated class encompassed a range of adulterant concentration – for which the PCA model captured the dominant directions of spectral variability among the mixtures. An important output of SIMCA is the distance of each sample to each class model, usually summarized in Coomans plots (Figure 3).

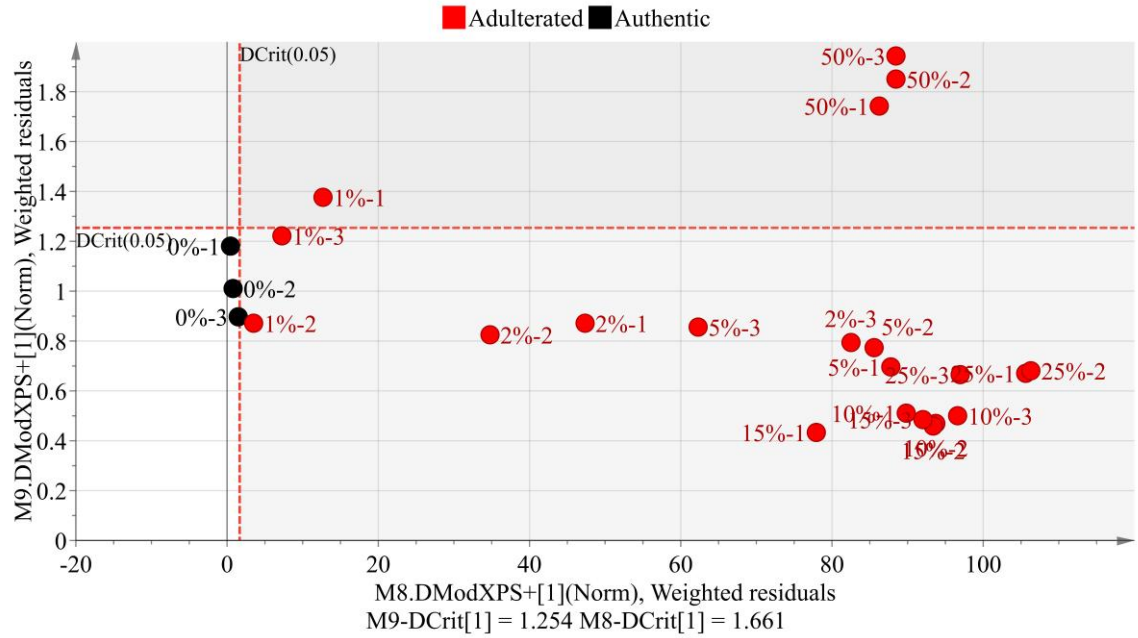

Figure 3. Coomans plot for SIMCA classification of gambir samples based on FTIR–ATR spectral data. The plot shows the distance of samples to the Authentic class model (x-axis) versus the distance to the Adulterated class model (y-axis), with 95% confidence acceptance regions indicated for each class.

Our SIMCA results demonstrated clear separation between the authentic and adulterated gambir classes. In the Coomans plot (distance to Class 1 vs. distance to Class 2), the authentic gambir samples (black points) fell well inside the acceptance region of the authentic gambir model and far from the adulterated class model. Conversely, all intentionally adulterated samples (red points) were positioned close to the adulterated class model and beyond the threshold distance (D_{Crit}) for the authentic class. This indicates that none of the adulterated samples were misclassified as "authentic." The model effectively recognizes even the lowest level of adulteration (1%) as not belonging to the authentic gambir class. We note that the 1% samples had the smallest distance from the authentic class boundary (sometimes very close to the 95% confidence limit), reflecting their strong similarity to authentic gambir. Nonetheless, they were still correctly differentiated.

These findings suggest that SIMCA modeling on the FTIR spectral data can serve as a reliable one-class classifier to flag gambir samples that deviate from the authentic profile. In practical terms, a SIMCA model built on known authentic gambir could be used for routine screening: any new sample falling outside the confidence ellipsoid of the "authentic" PCA model would be suspected as adulterated. Our work provides a proof-of-concept for this approach in gambir quality control.

PLS-R Quantification of Adulterant Concentration

The most significant outcome of this research is the successful development of a PLS regression model to quantify the adulterant concentration in gambir from its IR spectrum. Using the training set (0–50% adulterant concentration) spectra and their known concentrations, we built a calibration that related spectral variations to % adulterant concentration (Figure 4).

The optimized PLS-R model used a small number of latent factors (determined by cross-validation; in our case, the optimal number of components was found to balance high R^2 with low prediction error). The regression of predicted vs. reference concentration showed an excellent fit. The **coefficient of determination (R^2)** for the calibration was 0.9884, indicating that 98.84% of the variance in adulterant concentration could be explained by the IR spectral data in the model.

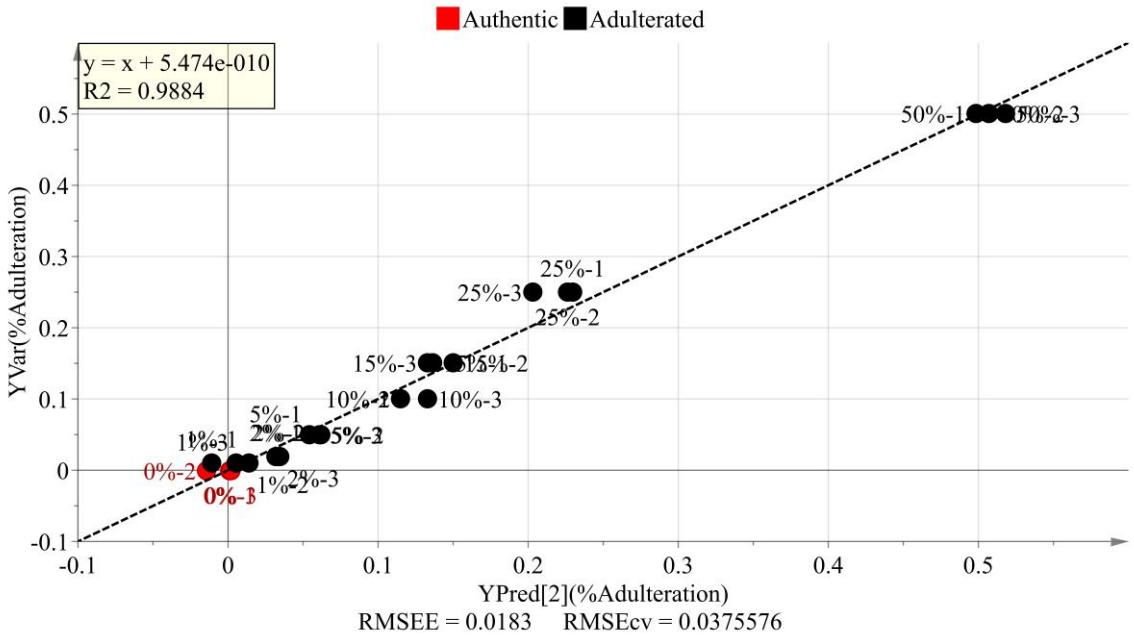

Figure 4. Observed (y-axis) vs. predicted (x-axis) adulteration percentages for the PLS-R calibration of the model. The line represents the ideal y=x correlation.

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This is an extremely high R^2 for a calibration model, reflecting the strong correlation between spectral changes and adulterant concentration across the range tested. The calibration curve was nearly a straight line with a slope close to 1, and points clustered tightly around the line, further illustrating the model's accuracy.

The **error metrics** for the model were also very favorable. The root mean square error of calibration (RMSEC) was 0.0183 (in fraction of adulterant, equivalent to ~1.83% adulterant concentration), and the root mean square error of cross-validation (RMSECV) was 0.0375 (~3.75% adulterant concentration). These low error values suggest that the model can predict adulteration level with only a few percent uncertainty. Additionally, the small difference between RMSEC and RMSECV implies the model is not overfit and will generalize well to new data (a large gap would indicate overfitting). According to chemometric guidelines, a good PLS model should have high R^2 and low RMSE values, and our model meets these criteria.

From the calibration model, we derived the Limit of Detection (LOD) and Limit of Quantification (LOQ) for adulterant in gambir. Based on the calibration slope and noise level (following a standard formula using the residual standard deviation), the LOD was calculated at 5.78% adulterant concentration and the LOQ at 17.50% adulterant concentration. These values mean that adulterant concentration as low as around 5% could be detected (though not precisely quantified), and above ~17.50% could be quantified with acceptable accuracy and precision. Notably, detecting ~5% adulteration in an herbal product by chemical analysis is quite sensitive – this demonstrates the power of the FTIR-chemometric approach. Many traditional chemical assays might not easily catch such low-level adulteration without extensive sample processing. Our method achieves it rapidly on the basis of spectral fingerprint differences.

To validate the PLS-R model, we applied it to the five test set samples (which were not used in model building) (Figure 5). The model's predictions for the four prepared test adulteration samples were very close to their true values. For instance, the 0%, 1%, 15%, and 50% test samples were predicted with minimal error (the predicted values deviated by only about 0–2 percentage points from the actual, which is within the model's error range). This confirms that the model can accurately interpolate within the calibration range.

Finally, we applied the PLS-R model to the market gambir sample (the unknown real-world sample). The model predicted ~25% adulterant concentration for this sample. This is a striking result – it suggests that the gambir purchased from the market contained nearly one-fourth foreign material (likely fillers similar to our SP36 adulterant).

Table 2. PLS-R Model Performance for Adulterant Quantification in Gambir.

Performance Metric	Value
Calibration R^2	0.9884
RMSEC (Calibration error)	~1.83% adulterant concentration
RMSECV (Cross-validation error)	~3.75% adulterant concentration
LOD (Limit of Detection)	~5.78% adulterant concentration
LOQ (Limit of Quantification)	~17.50% adulterant concentration

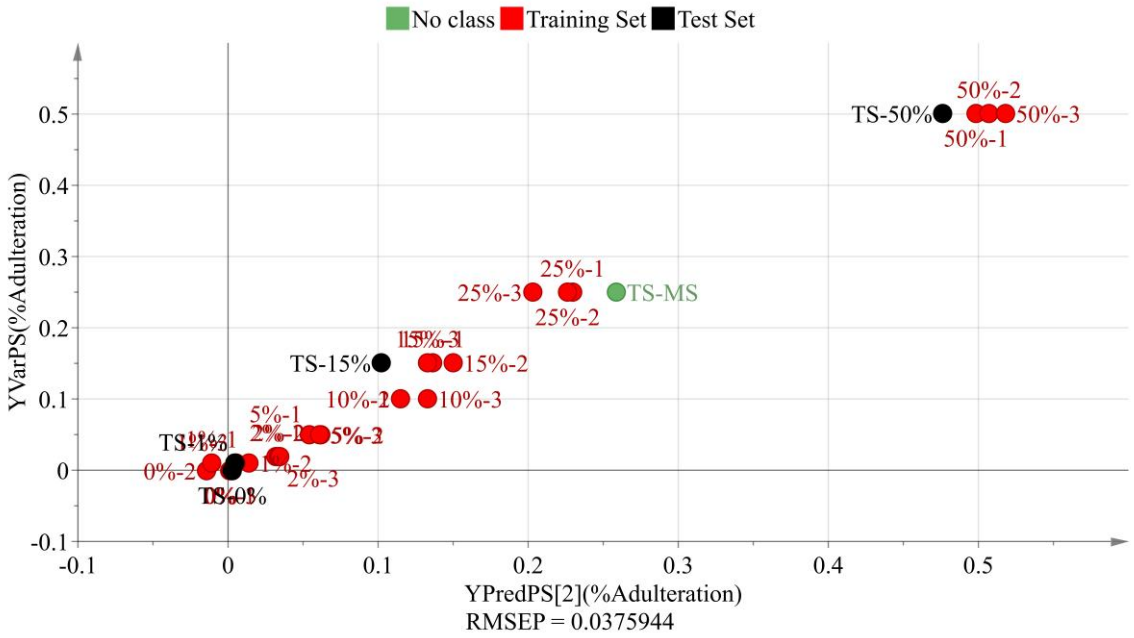

Figure 5. Predicted (x-axis) vs. actual (y-axis) adulteration percentages for both training set (red dots) and test set samples (black dots) using the PLS-R model. The market sample (green dots) prediction is also shown.

While we did not have an independent analytical method to verify this number, the model's robustness and the clear spectral differences give credence to the prediction. This finding highlights that adulteration of gambir is indeed occurring in the supply chain and can reach significant levels. For context, a 25% adulteration would substantially degrade the quality of gambir, underscoring the importance of routine authenticity testing.

In summary, the FTIR-based PLS regression provided a quantitative tool to complement the qualitative classification. Together, our results demonstrate a comprehensive solution: SIMCA models for identifying adulterated samples and PLS-R for determining how many adulterant concentration is present. Our work adds to this body of evidence by providing the first such study focused on gambir, an important herbal product. It not only confirms that adulteration can be detected and measured in gambir, but also establishes a framework that could be applied to other herbal materials facing similar issues of fraud.

Compared to similar studies on herbal product adulteration, the sensitivity and accuracy achieved by our method are noteworthy. For instance, infrared spectroscopy-based methods for honey authentication typically achieve high classification accuracy only when adulterant levels exceed roughly 5–7%[16]. In the case of black pepper, economically motivated adulteration (e.g., with papaya seeds or spent material) is usually considered significant at >10% dilution, and IR fingerprinting methods readily distinguish such adulterated samples[14]. In our study, the ATR-FTIR chemometric approach could reliably detect ~5% adulteration in gambir, indicating a higher sensitivity than many prior reports for other products. In terms of quantitative performance, our PLS-R model's R^2 (~0.99) and low error margins are comparable to or better than those reported in similar adulteration quantification studies. For example, FTIR/chemometric models developed for quantifying adulterants in essential oils and olive oil have achieved calibration R^2 values in the 0.95–0.99 range with prediction errors of a few percent[13],[33]. Thus, our results align well with the state-of-the-art in this field, and demonstrate that ATR-FTIR spectroscopy combined with multivariate analysis can provide extremely sensitive and accurate detection of adulteration in herbal products.

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It should be noted, however, that the external validation of our models was limited. We tested the method on a small set of independent samples – including only one type of adulterant (phosphate fertilizer) and one real market sample. Therefore, the generalizability of the models to other adulterants or to gambir samples from different sources has not yet been established. Future studies should expand the validation by applying this approach to a larger number of gambir samples from various regions and incorporating other potential adulterants (e.g., starch, clay, other fillers) to further challenge the model. Despite this limitation, our current findings provide a strong proof-of-concept that ATR-FTIR spectroscopy coupled with chemometrics can be an effective tool for gambir adulteration detection. With broader validation, this method could be confidently adopted for routine screening and quality control in the gambir industry.

Conclusions

This research demonstrates that a combination of FTIR spectroscopy and chemometric analysis is a powerful approach for detecting adulteration in gambir. The ATR-FTIR spectral fingerprints of authentic versus adulterated gambir contain sufficient information to both recognize the presence of foreign substances and quantify their amount when analyzed with multivariate models. Specifically, the SIMCA classification modeling on FTIR data was able to differentiate authentic gambir samples from those adulterated with a fertilizer-based contaminant, effectively flagging even low levels (~5%) of adulteration. Moreover, the PLS regression model provided an excellent quantitative calibration, achieving a high correlation ($R^2 \approx 0.988$) between spectral data and adulterant concentration and enabling prediction of adulterant levels with low error. The sensitivity of the method was high, with a detection limit on the order of 1% adulterant. When applied to a real market sample of gambir, the model predicted about 25% adulteration, revealing a significant authenticity issue in that sample.

In summary, ATR-FTIR spectroscopy combined with chemometric modeling proved to be a rapid, nondestructive, and sensitive method for gambir authentication. Even low-level adulteration could be identified and quantified, making this approach suitable for routine quality control. It can be implemented by producers or regulators as a screening tool – for example, using portable FTIR devices to test gambir batches – in order to ensure product integrity and compliance with quality standards. While further validation on more samples is recommended, this study provides a foundation for safeguarding gambir and other herbal products from adulteration through advanced spectral fingerprinting techniques.

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Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

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Author Contributions

D.S.: Conceptualization, corresponding author responsibilities, and supervision. Y.E.: Methodology, investigation, data collection, formal analysis, data curation, visualization, and writing —original draft preparation. A.A.: Methodology, validation, and writing —review and editing.

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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