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Research Article

Infrared Spectroscopy (FT-NIR) and t-Distributed Stochastic Neighbor Embedding (t-SNE) as an Analytical Methodology for Rapid Identification of Tea Adulteration

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Teas and infusions are beverages obtained from plant extracts of flowers, fruits, stems, roots, and other parts of plants, after undergoing a process of selection, cleaning, sanitization, and dehydration. Historically used for medicinal purposes, these beverages are appreciated for their pleasant flavor and aroma, stress reduction, calming and digestive effects, and as an aid in the composition of special diets, in addition to being an excellent option as a social drink at various types of events. It is not uncommon to find commercial tea preparations mixed with leaves and parts of other plants to increase profit and production volume, which constitutes fraud. The aim of this study was to perform FT-NIR spectroscopic characterization of leaves and pieces (petiole and stem) of three types of medicinal plants (Chamomile, Ginseng, and Quebra-pedras) used in the preparation of teas. Three cluster analysis methods were used to evaluate the ability of FT-NIR to identify plant types, with t-SNE presenting the best discriminatory power. The deconvolution of the spectra showed that fifteen vibration bands allow a good characterization of the samples, all with R^2 greater than 0.99. The presented approach has a great potential for quality control by industry and government agencies.

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1. Introduction

Many beverages are made from extracts of fruits, flowers, stems, roots, seeds, and other parts of plants. Examples include coffee, fruit juices, and teas. Among these beverages, infusions and teas are appreciated not only for their pleasant taste and aroma but also for their therapeutic and medicinal effects. Calming teas for stress reduction and improving sleep quality are easily found in pharmacies, supermarkets, and health food stores, as well as those with pain relief, anti-inflammatory, diuretic, anti-diabetic, anti-hypertensive, and functional properties[1][2][3][4].

The perception of the effects that teas have on the body has an important relationship with the culture and dietary intake of some ancient peoples, particularly the Chinese, with historical records of tea consumption dating back approximately 3,000 years[5][6][7]. With the development of scientific methods, teas began to be studied in more detail, and their health benefits were confirmed over time, with proof of functional properties, identification of bioactive compounds, and biomolecules of pharmacological interest[8][4][9][10].

Food product fraud is a very common practice, intentionally carried out to obtain increased profits, greater production volume, and unfair competition with competitors, characterizing a criminal act against consumer health and rights[11][12]. To combat this, many analytical techniques have been used to detect and quantify food adulteration, including teas and infusions. The most common methods are chromatography (liquid and gas), electrophoresis, mass spectrometry, fluorescence, X-ray, UV-visible and infrared spectroscopy, as well as numerous other traditional techniques, such as thermogravimetric analysis, volumetric titration, refractometry, and analysis of the electrical properties of materials[11] (Kennedy et al., 2021).

FT-NIR spectroscopy stands out because it offers a short analysis time, low operating cost, repeatability, accuracy of results, absence of preparatory steps for the material to be analyzed, non-destructiveness, and the fact that it does not use chemical solvents or toxic compounds, common in traditional analyses[13][14][15]. Similar to other food products, the raw materials used in the production of teas and infusions must be classified, identified, and properly characterized to avoid fraud and adulteration[11].

The objective of this study was to use Fourier Transform Near-Infrared Spectroscopy (FT-NIR) to characterize and identify the vibrational bands related to the main chemical groups present in the leaf, petiole, and stem of three types of medicinal plants used in the production of teas. The analyses were performed for Chamomile (*Matricaria recutita* L.), Ginseng (*Panax Ginseng*), and Quebra-pedrass (*Phyllanthus niruri*). In addition to the graphical analyses of deconvolutional spectroscopy and derivative spectroscopy, three cluster analysis methods were tested. It is important to highlight that t-distributed Stochastic Neighbor Embedding (t-SNE) is a nonlinear statistical method used for visualizing high-dimensional data, widely applied in the analysis of complex data, with a large amount of information, and generally presenting better performance than Principal Component Analysis (PCA), which is a linear statistical method aimed at reducing the number of dimensions while maintaining the variance of the data set. Along with t-SNE and PCA, a dendrogram was created—a diagram obtained from a cluster analysis—which, like the others, aimed to evaluate the potential of using FT-NIR to classify and group tea raw materials.

2. Material and Methods

FT-NIR analyses were performed at the Materials Spectroscopy Laboratory of the Physics Department of the Federal University of Juiz de Fora, MG.

2.1. Sample Collection and Preparation

The Chamomile, Ginseng, and Quebra-pedras samples were prepared at the Food Microbiology Laboratory of the Department of Food Science, DCA/UFLA. Three dehydrated samples of each type of plant were collected, duly identified, and labeled as R1, R2, and R3. A fraction composed of petioles and leaf stems was also used for each type of plant, labeled as 'piece'. All samples were sieved through a coarse sieve (mesh 10), and a mass of (0.200±0.005) grams was measured from each one and reserved for FT-NIR analyses. All analyses performed with FT-NIR were done in triplicate, with different readings taken from different portions of the analyzed material.

2.2. FT-NIR Analyses

Analyses of the samples were carried out with the Multi Purpose FT-NIR Analyzer from Bruker, operating in reflectance mode in the range of 12,000 to 4,000 cm^{-1} wavenumbers with a Te-InGaAs detector and 4 cm^{-1} resolution. The OPUS® software version 5.5 was used for data acquisition. The samples were placed in borosilicate cuvettes with 8 mm thickness. Each analysis was performed in triplicate with 64 scans for both simulated and control samples.

2.3. Statistical Analyses and Treatment of Experimental Data

Initially, the FT-NIR spectra were normalized to the range 0 to 1 using the SAS OnDemand statistical package. Fityk software, version 1.3.1, was used for baseline correction, derivative spectroscopy analyses, and deconvolution of the spectra.

The cluster analyses were performed in the Orange Data Mining software, version 3.37.0, using the PCA (Principal Component Analysis), t-SNE (t-distributed Stochastic Neighbor Embedding), and dendrogram modules.

LibreOffice Calc software, version 24.2.5, was used to tabulate the data. The graphs were created in the SciDAVis software, version 2.8, and edited in the Inkscape software, version 1.3.2, for color enhancement.

3. Results and Discussion

3.1. FT-NIR Derivative Spectroscopy

Figure 1(a) presents the normalized FT-NIR reflectance spectra of all samples in the experiment. This figure shows that the FT-NIR spectra of the samples do not overlap throughout the analyzed frequency range, indicating that near-infrared spectroscopy is a potential technique to characterize and detect differences between the plants studied based on the vibrational characteristics of the chemical groups present in their composition. Figures 1(b) and (c) refer to the derivative analysis of the spectra and show that for the same plant, its chemical composition is very similar among the different experimental replicates of the dehydrated leaves, as their reflectance bands occur practically at the same frequencies of the spectrum, and with approximately equal amplitudes. The same is not observed for the "piece," a material composed of leaf petioles and stems, whose vibration bands and intensities are different from those observed in the infrared spectra of the leaves. Thus, it is possible to identify commercial teas that have been intentionally tampered with by the addition of leaf petioles and stems to adulterate the product. The same pattern is observed in the other derivative spectra for the Ginseng and Quebra-pedras samples.

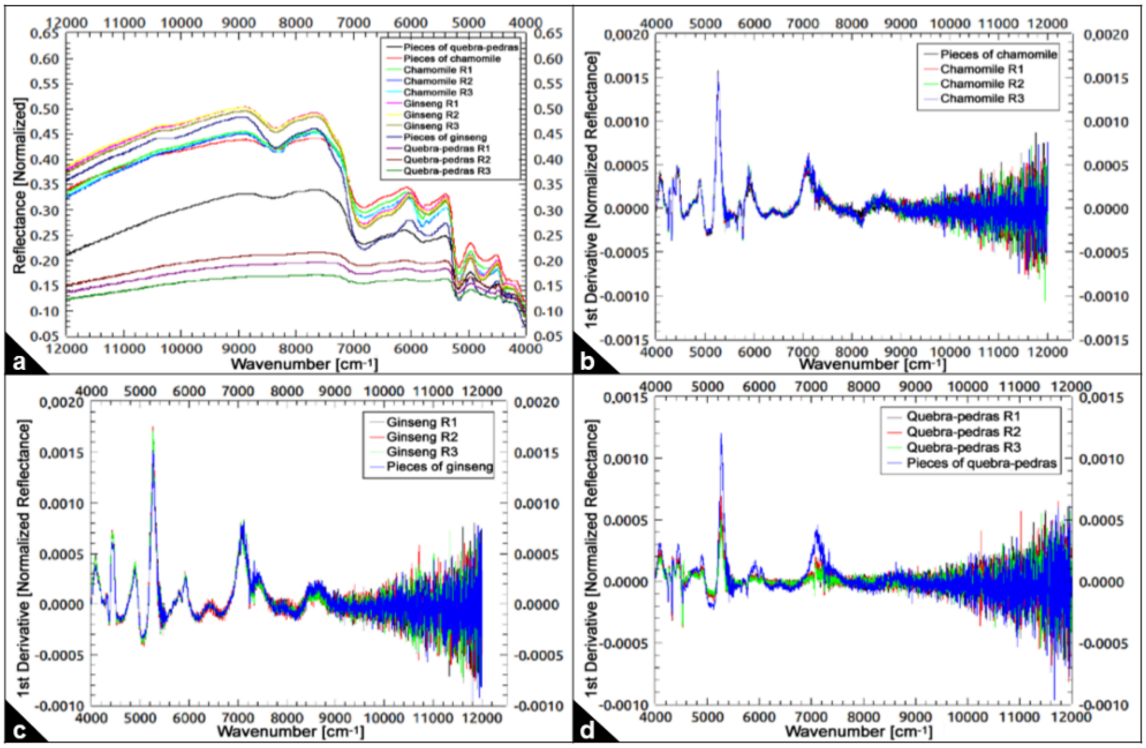

Fig. 1. In (a), the normalized FT-NIR spectra for all samples are shown. In (b), the first derivative of the normalized FT-NIR signals for Chamomile (leaves and pieces) is presented. In (c), the first derivative of the normalized FT-NIR signals for Ginseng (leaves and pieces) is displayed, and in (d), the first derivative of the normalized FT-NIR signals for Quebra-pedras (leaves and pieces) is illustrated.

3.2. Deconvolutional Spectroscopy

3.2.1. Analysis of Chamomile Spectra

Some chemical compounds present in Chamomile are reported by Oliveira[16]: chamazulene, beta-farnesene, alpha-bisabolol oxide A, alpha-bisabolol oxide B, alpha-muurolol, spathulene, beta-eudesmol, E-spiroether, hexadecanoic acid, E, E-alpha-farnesene, and nonacosane. These compounds have functional groups that vibrate at different frequencies in the FT-NIR spectrum, as shown in Figures 2(a) to 2(c). The chemical composition observed among the different repetitions is quite similar, with bands that have approximately the same amplitude and the same wavenumbers. Some groups, however, do not follow this pattern, such as the band presented at 10,340.70 cm^{-1}, which does not appear in Figure 2(a) but is present in the other figures, including (d), corresponding to the spectrum of leaf petioles and stems. This region of the spectrum (10,340.70 cm^{-1}) is associated with the vibration of functional groups that have C-H bonds, including C=C-H of alkene groups and aromatic rings, and C-C-H bonds, associated with cycloalkanes and aliphatic chains of organic compounds[17].

The band near 4703.34 cm^{-1} that appears in Figures 2(a) and (d) is also not present in the other spectra, (b) and (c), indicating that the chemical composition of tea may undergo small variations depending on the part of the plant used (leaves or petioles and stems). This band is typical of stretching vibrations of the O-H group, which are very common in the alcohol groups (ROH) present in essential oils[18].

In Figures 2(a) to (d), the deconvolution of the spectra shows high values of R2R^2R2 (coefficient of determination) and low values of WSSR (weighted sum of squared residuals), indicating an excellent representation of the original spectrum by means of Gaussians.

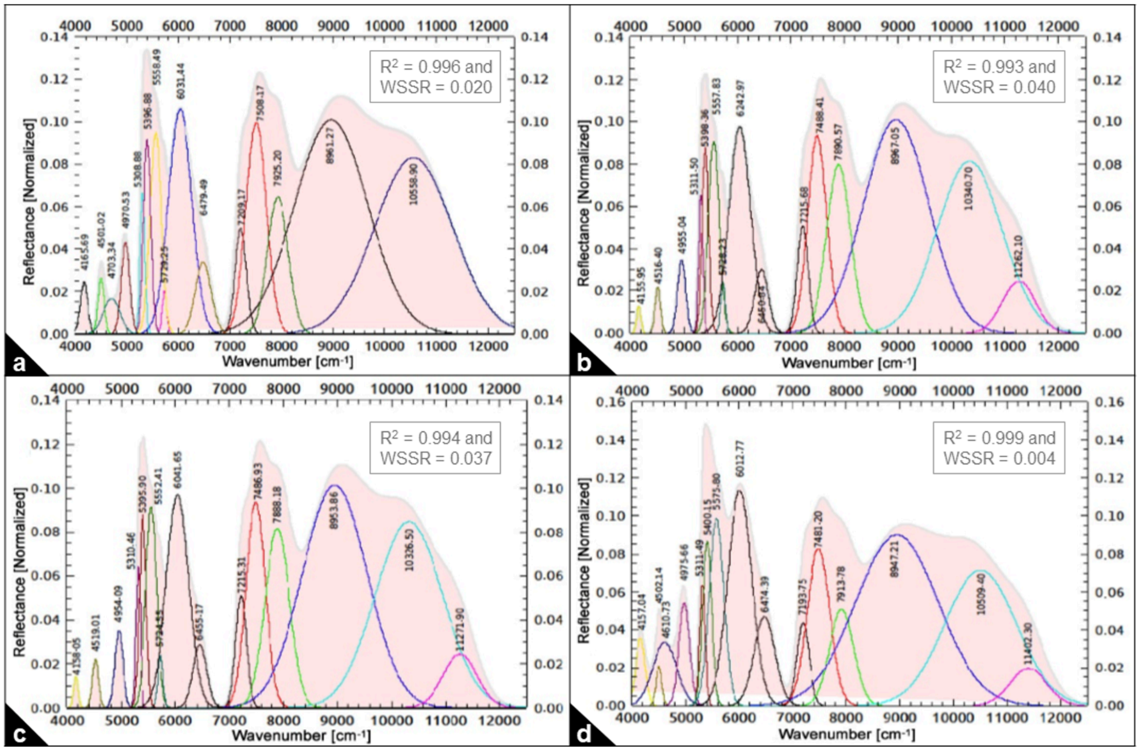

Fig. 2. In Figures (a) to (c), the normalized deconvolution spectra of Chamomile leaves for repetitions 1 to 3, respectively, are shown. In (d), the normalized deconvolution spectrum of the pieces (petiole + stem) of Chamomile leaves is presented.

3.2.2. Analysis of Ginseng Spectra

Ginseng is a plant known as the “king of herbs,” a title attributed to its wide variety of active compounds with medicinal effects and potential health benefits[19][20][21]. Among the bioactive compounds reported in the literature are ginsenosides, polysaccharides, volatile oils (essential oils), alkaloids, and amino acids, which are associated with antioxidant, anti-cancer, anti-inflammatory, weight control, anti-stress, and anti-aging effects, among others[19][22][23].

In terms of infrared reflectance, the analyzed samples do not present significant spectral discrepancies, with similarities in the positions and amplitudes of all vibration bands, except for the spectrum in Figure 3(d), which refers to the petioles and stems of the leaves. The band amplitudes in this spectrum are visibly different from those observed for the leaf samples. The three bands with the largest area under the Gaussian are at 7524.23 cm^{-1}, 8753.56 cm^{-1}, and 10,367.10 cm^{-1}, respectively. In the region close to 7524.23 cm^{-1}, C-H bonds vibrate, associated with the presence of alkyne and alkene groups. Near 8753.56 cm^{-1} is a region associated with the vibration of the carbonyl group (C=O), present in various organic compounds such as esters, aldehydes, and ketones. The band near 10,367.10 cm^{-1} corresponds to vibrations of the C-H group, associated with alkanes, alkenes, cycloalkanes, and several other organic compounds[24].

In Figures 3(a) to (d), the deconvolution of the spectra shows high values of R2R^2R2 (coefficient of determination) and low values of WSSR (weighted sum of squared residuals), indicating an excellent representation of the original spectrum by means of Gaussians.

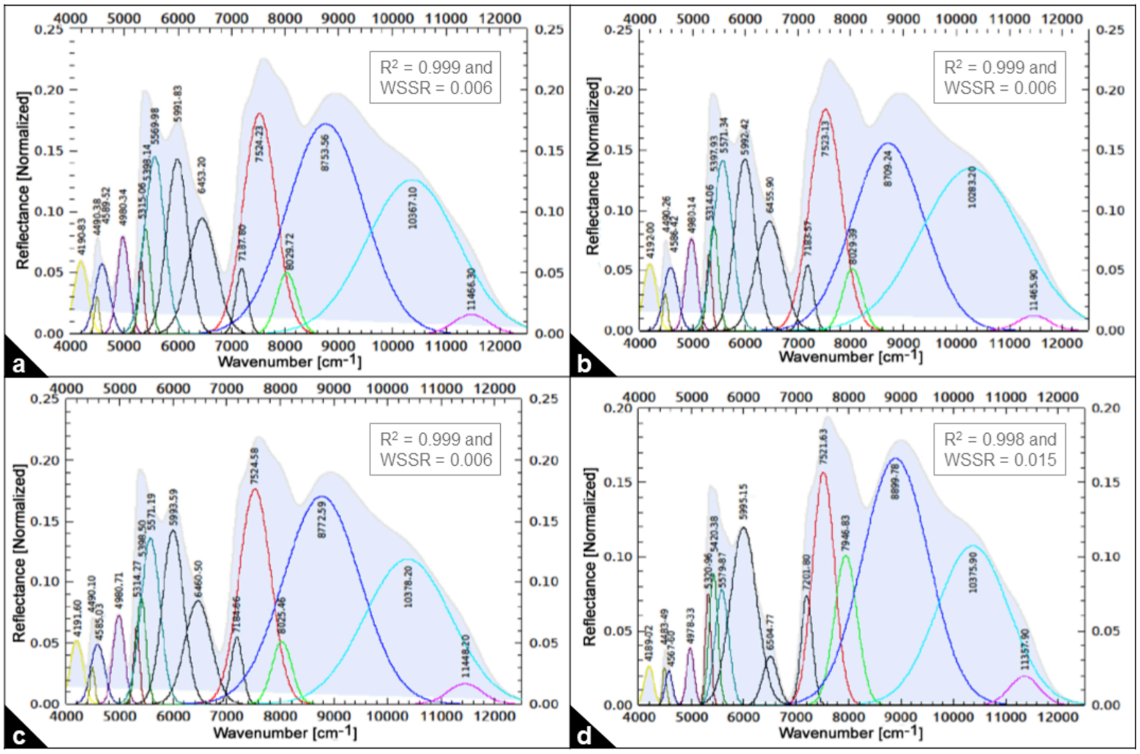

Fig. 3. In Figures (a) to (c), the normalized deconvolution spectra of Ginseng leaves for repetitions 1 to 3, respectively, are shown. In (d), the normalized deconvolution spectrum of the pieces (petiole + stem) of Ginseng leaves is presented.

3.2.3. Analysis of Quebra Pedras Spectra

Methyl salicylate, flavonoids, phyllanthin, phyllocrisin, filavin, cineole, cymol, linalool, securimine, filatidine, and salicylic acid are chemical compounds present in stone breaker, as reported by Embrapa[25]. Functional and bioactive attributes include hepatoprotective and antioxidant activity, described by Sanjeev Khanth et al. (2023), while antidiabetic potential is reported by Vinay Bhushan et al.[26].

The quebra pedras leaves did not present a homogeneous spectral pattern, as observed when comparing Figures 4(a) and (b), particularly regarding the amplitude of the signals. The band in the region 12,055.70 cm^{-1} is present in Figures 4(b) and (d) but absent in the other samples. This region corresponds to the vibration of N-H groups, indicating the possible presence of compounds containing amine and amide groups, such as nirurin and phyllanthin present in the plant[27]. The bands with large areas under the Gaussian, in the region close to 9947.94 cm^{-1}, in the spectra of all samples in Figure 4, refer to characteristic vibrations of C-H bonds, which are present in many organic compounds, especially short-chain alkanes, cyclohexane, cycloalkanes, and alkenes[17]. This band has a proportionally larger area in the petiole and stem sample of stone breaker compared to the leaf samples.

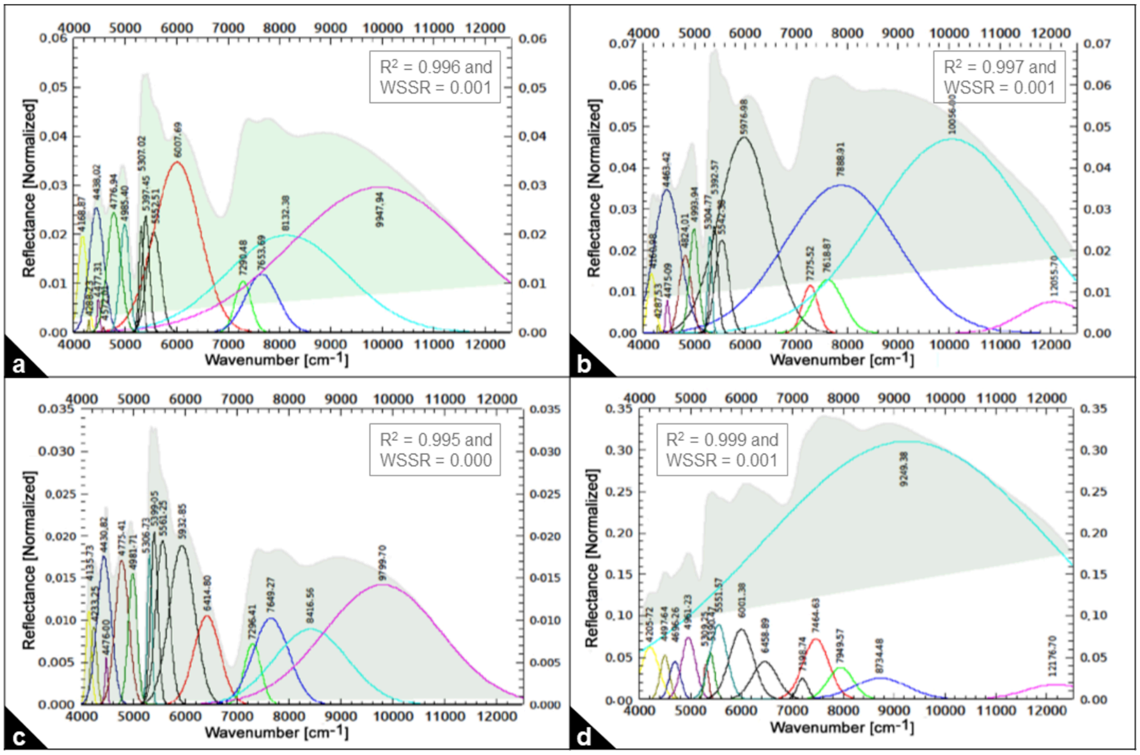

Fig. 4. In Figures (a) to (c), the normalized deconvolution spectra of Quebra-pedras leaves for repetitions 1 to 3, respectively, are shown. In (d), the normalized deconvolution spectrum of the 'pieces' (petiole + stem) of Quebra-pedras leaves is presented.

In each of the deconvolution graphs presented in Figures 2, 3, and 4, a total of 15 Gaussians were fitted to represent the entire normalized spectrum. All fittings had an R^2 value greater than 99%, with a low error, as indicated by the WSSR (weighted sum of squared residuals) parameter.

3.3. *Cluster Analysis of Teas*

The cluster analysis presented in Figures 5(a) and (b) shows that both techniques used, PCA and t-SNE, are effective in representing FT-NIR spectral differences in a two-dimensional space, with minimal loss of information. This conclusion is based on the percentage values of variance representation observed in the PC1 (89.86%) and PC2 (9.03%) axes, which together account for 98.89% of the total variance for these first two principal components. t-SNE proved to be the most efficient in forming and identifying groups, possibly due to its non-linear clustering approach that considers spectral features not detected by PCA. Thus, it can be inferred that plant samples not fitting within the regions (ellipses) presented in Figures 5(a) and (b) may potentially be considered fraudulent, as their FT-NIR spectra do not match the patterns obtained for the leaves and pieces of Chamomile, Ginseng, and Quebra-pedras.

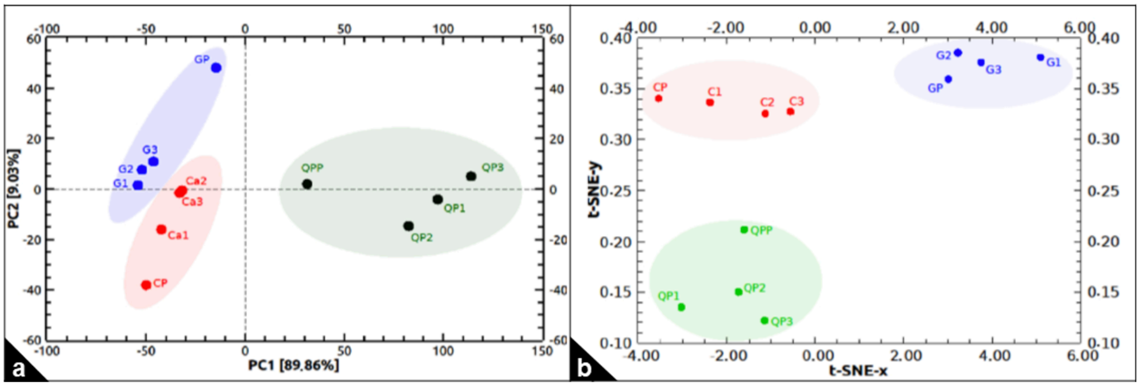

Fig. 5. In (a), the principal component analysis (PCA) of all Chamomile, Ginseng, and Quebra-pedras samples is shown. In (b), the t-SNE plot of all Chamomile, Ginseng, and Quebra-pedras samples is displayed. In the figures, points with 'G' as the first letter refer to Ginseng samples, 'C' as the first letter refers to Chamomile samples, and 'Q' as the first letter refers to Quebra-pedras samples. The subscripts 1, 2, and 3 correspond to samples 1, 2, and 3, respectively, while the subscript 'P' indicates piece.

The dendrogram in Figure 6, obtained using a linear clustering method, demonstrates that it is possible to group samples based on their similarity using FT-NIR spectroscopy data. FT-NIR can detect the presence of key chemical groups, allowing for the classification of samples into different categories. Leaves and plant parts are also placed into distinct groups. By considering a cut line at 30 Euclidean distance units (vertical dashed line in Figure 6), the result allows for the grouping of plant parts (stems and petioles) into categories different from those formed exclusively by leaves.

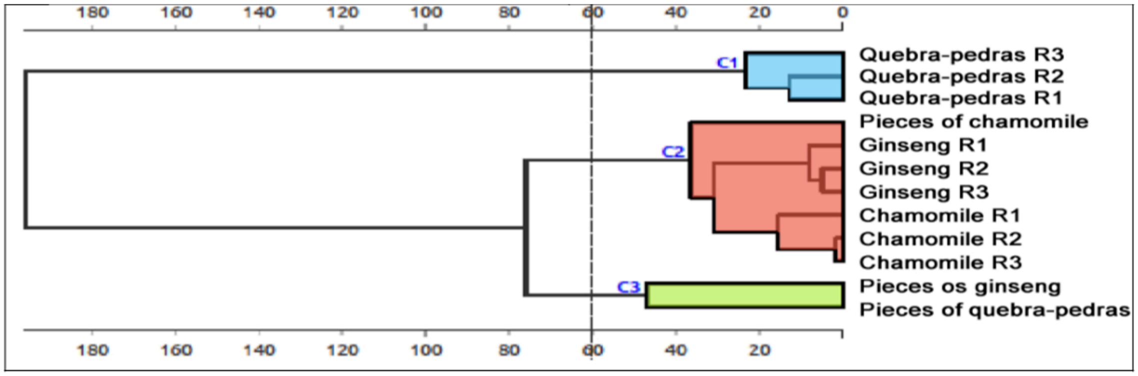

Fig. 6. Dendrogram of the cluster analysis performed based on the normalized FT-NIR spectral signals of all Chamomile, Ginseng, and quebra pedra samples.

4. Conclusion

This study demonstrated that the combination of FT-NIR spectroscopy and multivariate analysis techniques constitutes an analytical methodology capable of forming groups within which the spectroscopic and vibrational characteristics that define the chemical markers of the medicinal plants under study are preserved. This is important because spectra obtained from complex samples or those that do not belong to these groups clearly indicate possible adulteration, suggesting fraud. It was possible to differentiate samples obtained from leaves from those obtained from leaf stems and petioles (pieces). Derivative spectroscopy was used as an exploratory investigation technique, allowing the identification of frequency bands with the greatest indications and evidence of differences between the samples studied. Among the grouping methods used, t-SNE showed the best separation capacity between the samples, as the samples formed graphically well-defined and distant groups, making their identification unequivocal. The deconvolution of the spectra showed that a total of fifteen Gaussians was sufficient to characterize the main vibration frequencies of the chemical groups present in the samples. These frequencies represent a variety of vibrational modes, such as bond vibrations, combinations, and overtone bands. The study constitutes an approach that has great potential for quality control by industries and government agencies.

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Declarations

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