Feasibility Assessment of the Identification of the Source of Condensed Tannins in Leathers by FTIR Spectroscopy and Chemometrics

by

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Abstract

This study aimed to investigate the feasibility of identifying the type of plant used in the tanning of leathers by cost-effective Fourier transform infrared (FTIR) spectroscopy. The investigation was performed on European horse-chestnut (fruit peel), mimosa, and quebracho and three specimens of mimosa-tanned leathers. Tannin extraction from plants was performed in an ultrasonic bath using acetone-water solvent (70%). Tannin extraction from leathers was carried out from the corium fibers using acetone-water solvent (1:1). After extraction, filtration, centrifugation, and solvent removal, the samples were subjected to FTIR spectroscopy. Principal Component Analysis (PCA) and hierarchical clustering were used to identify the source of tannins based on FTIR results. In addition to FTIR spectra, their first and second derivatives were also used in statistical analyses. The obtained FTIR spectra and their derivatives and the results of PCA and hierarchical clustering showed that rich plant sources of condensed tannins can be well differentiated by spectroscopy in the fingerprint region (700-1800cm^{-1}). The PC1-PC2 plot in the analysis of FTIR spectra and the PC2-PC3 plot in the analysis of derivatives showed the best ability to differentiate and identify the extracts. Multivariate PCA and cluster analyses performed well in identifying the type of plant used in the tanning of the studied leathers, especially when applied to the derivatives of FTIR spectra.

Introduction

Tannins are polyphenolic compounds produced in plants as secondary metabolites.[1] The synthesis and accumulation of these compounds are influenced by a variety of factors including photosynthesis, season, temperature, and rainfall.[2] Tannins can be extracted from various parts of almost all plants including roots, stems, leaves, and secretions. Tannins are generally classified into two groups: hydrolysable and condensed. Hydrolysable tannins themselves can be divided into two sub-groups: gallotannins and ellagitannins. Hydrolysable tannins are extracted from plants like chestnut, sumac, tara, and myrobalan. Sources of condensed tannins include mimosa, quebracho, hemlock, alder, willow tree, and gambir.[3,4] Condensed tannins, which classify as naturally occurring proanthocyanidins, are polyflavonoids composed of a chain of flavan-3-ol units. The most common proanthocyanidins are those composed of catechin or epicatechin chains interlinked by C4 → C6 or C4 → C8 bonds.[5] These tannins are typically joined by C4 → C8 bonds, but this may also occur through C4 → C6 bonds.[6]

Because of their relatively high molecular weight (between 500 and 3,000 Daltons),[7] tannins can form strong complexes with carbohydrates and proteins.[8] The oldest application of plant tannins was in the tanning of rawhide to stabilize animal skin proteins against decay.[3] Plant tannins have generally been the most commonly used group of tanning agents in leather processing before the nineteenth century.[4,9] However, the choice of the type of plant used in the tanning process could be influenced by a variety of factors including climate, predominant vegetation, culture, availability, economic condition, target quality, and target application.

Therefore, one issue of concern in the study of leather products, especially historical leather artifacts, is how to identify the type of plant used in the tanning process; information that can provide valuable insights into the above-mentioned factors. Some efforts have been made in this regard and there are indeed several reports of successful use of chromatographic and mass spectrometric methods for this purpose.[10-13] However, in addition to being quite expensive, these methods require extensive preparation and have their own limitations. In addition, identifying the type of tannin, and not the source of the plant used in tanning, has been one of the topics of interest to researchers. In previous studies, spectroscopic methods including FTIR and UV-Vis have been used to identify the type of tannin present in the leather structure.[14-16] Using these methods, it is indeed possible to well identify the type of tannin and determine whether it is condensed or hydrolysable (gallotannin or ellagitannin). In addition to spectroscopic methods, there are also reports of the successful use of various spot tests including ferric, vanillin, butanol acid, rhodanine, and nitrous acid tests to identify the type of tannin used in leathers.[6,17,18]

However, the great majority of studies on leather artifacts that have used spot tests and spectroscopic methods have been mainly focused on identifying the type of tannin used in tanning rather than the type of source plant. Research on tannins obtained from

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different plants has shown that spectroscopy-based chemometrics can be used to identify and classify extracts based on the type of source plant. These studies have generally used FTIR, UV-Vis, and NIR spectroscopy methods.[19-21] However, considering their good performance in classifying plant extracts, chemometric methods can also provide a good estimate of the source plant of tannins used in leathers through statistical analysis of spectroscopic data. In this study, the goal was to assess the feasibility of identifying the type of plant used in the tanning of a leather product through FTIR spectroscopy and chemometric methods. In the first phase of this feasibility study, the assessments are performed on tannins extracted from mimosa, quebracho, and European horse-chestnut and three specimens of mimosa tanned leather.

Materials and methods

Tannin extraction

Tannins used in the study were extracted from three plants, namely quebracho, mimosa, and European horse-chestnut (fruit peel). The three leather specimens used in the study were produced by tanning goat and sheep hides with mimosa tannins (tanned in 2016). The characteristics of the specimens are given in Table I.

For tannin extraction from plants, 10mL of acetone-water solution (70%) was added to 200mg of the powdered plant substance in a sealed container, which was then placed in an ultrasonic bath. The extraction process was performed using Makkar [22] method in two 10-minute intervals with a 5-minute rest. The extract was filtered by a Whatman filter paper grade 42, and the filtered extract was then centrifuged at 3000 rpm for 10 min. The centrifuged solution was separated and placed in an oven at 70°C for one hour for solvent removal.

Tannin extraction from leather was performed using water-acetone solution (1:1) and samples collected from collagen fibers of the reticular layer. For every 10 mg of leather fiber, 1 mL of the solution was added to the sample.[10,15] The extraction was performed in a sealed container at standard laboratory temperature over a period of 48 h, during which the samples were placed on a shaker. Afterward, solution filtering and centrifugation and solvent removal were performed in the same way as described in the previous paragraph for tannin extraction from plants.

FTIR spectroscopy and multivariate analysis

Fourier transform infrared spectroscopy was used to examine the molecular structure of the samples. For this purpose, tannins extracted were examined using the KBr pellet method. To make the plate, the extract and KBr powder were mixed in a ratio of 1:100 w/w. This analysis was performed using a Jasco 680-plus FTIR spectrometer (Jasco Inc., Japan). The spectra were recorded in 64 scans with a resolution of 2cm^{-1} in the range of 400-4000cm^{-1}.

Spectrum analysis was performed using the software Omnic9 and Originpro2021. After baseline correction, smoothing, and normalization to the 0-1 range, spectra first and second derivatives were obtained. Normalization of spectra intensity was performed to reduce the effect of tannin content in KBr plates. The spectra as well as their first and second derivatives were analyzed by Principal Component Analysis (PCA) and hierarchical clustering. In PCA, three components were extracted and the discriminatory power of different components in two dimensions was assessed using covariance and correlation matrices. Spectral analysis was performed in the fingerprint region (700-1800cm^{-1}).

Table I
Characteristics of the studied plants and leathers

Sample type	Source	Origin	Code
Plant	Mimosa	Italy	MI-N
	Mimosa	Italy	MI-Y
	Mimosa	Italy	MI-H
	Quebracho	South Africa	KE-N-1
	Quebracho	South Africa	KE-N-2
	European horse-chestnut (fruit peel)	India	PB-HEND-1
	European horse-chestnut (fruit peel)	India	PB-HEND-2
Leather	Goat Leather	Tabriz, Iran	L-G 1
	Goat Leather	Tabriz, Iran	L-G 2
	Sheep Leather	Tabriz, Iran	L-Sh

Results and discussion

First, FTIR spectra were used to investigate the presence of condensed tannins in the specimens. The presence of tannin can be detected by the appearance of absorption peaks at 1606-1615 assigned to aromatic ring stretch vibrations, 1507-1518 due to skeletal vibration of the aromatic rings, 1196-1211 and 1030-1043cm^{-1} assigned to stretching vibrations of the C–O bond. In addition to these peaks, which are related to the general structure of tannins, condensed and hydrolysable tannins also have their own characteristic peaks. More specifically, the presence of two peaks at 1704-1731cm^{-1}, assigned to carbonyl stretching vibrations, and 1317-1325cm^{-1}, assigned to the symmetric stretching of the C–O bond of the ester function, indicates a hydrolysable tannin, which will be a gallotannin if additional peaks appear at 1082-1088, 870-872, and 758-763cm^{-1} and an ellagitannin otherwise. FTIR spectra of condensed tannins show, in addition to the characteristic peaks of tannins, three absorption peaks at 1283-1288, 1155-1160, and 1110-1116cm^{-1} and two other weak peaks at 976 and 842-844cm^{-1}. These bands can be assigned to the ethereal C–O asymmetric stretching vibration arising from the pyran-derived ring structure.[3,14,15] FTIR spectra of the studied specimens in the 700-1800cm^{-1} range after baseline correction are shown in Figure 1a. Given the presence of characteristic absorption peaks of condensed tannins in these spectra, condensed tannins can be considered the dominant compound in the specimens.

PCA and Cluster plots obtained from the FTIR spectra in the 700-1800cm^{-1} range are shown in Figure 2. The purpose of PCA conducted based on PC1 and PC2 is to identify plant extracts (tannins) that are chemically similar to each other, which makes it possible to differentiate and determine the types of source plants based on the

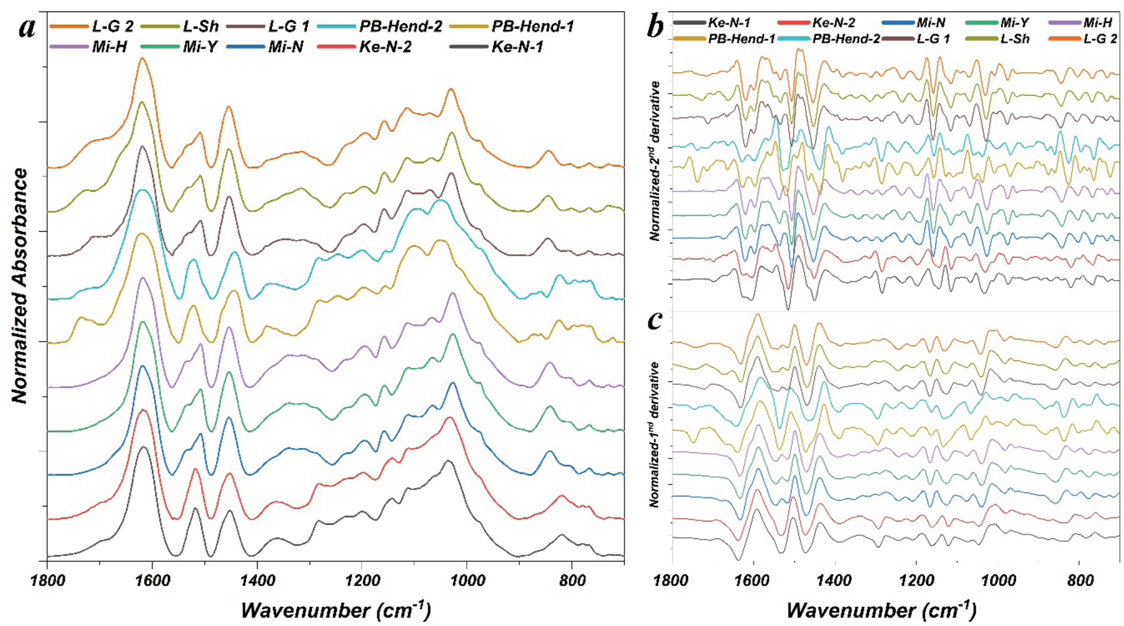

Figure 1. FTIR spectra of the condensed tannin extracts obtained from plants and leathers in the 700-1800cm^{-1} range after baseline correction **(a)**, the first derivative of the spectra **(b)**, the second derivative of the spectra **(c)**

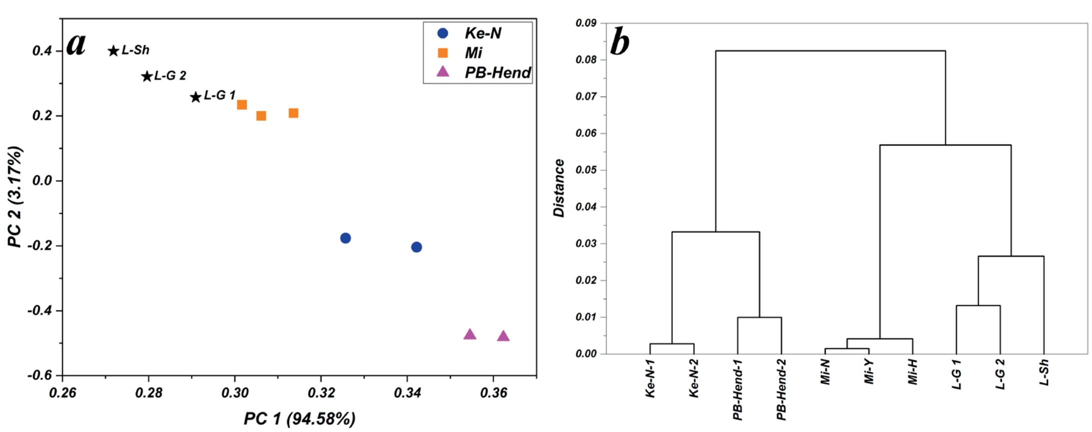

Figure 2. PCA plot **(a)** and hierarchical clustering plot **(b)** for the FTIR spectra of the condensed tannin extracts obtained from plants and leathers in the 700-1800cm^{-1} range

extracts. PCA results showed that tannins extracted from leathers have significant structural similarity to the one obtained from the mimosa plant. This is also well illustrated in the hierarchical clustering plot. These results indicate that among the studied plants, mimosa is most likely to be the source plant of the tanning agent used in the studied leathers.

The first and second derivatives of the spectra are displayed in Figures 1b and 1c. Using these derivatives, the differences and similarities of the spectra can be examined more closely with due consideration of overlapping peaks. Performing multivariate analyses on these derivatives results in more accurate differentiation and identification of compounds. PCA and Cluster plots obtained for the first and second derivatives of the spectra are shown in Figure 3. While for the spectra themselves PC1-PC2 provided good classification performance, for the first and second derivatives of the spectra, the best performance was observed in the PC2-PC3 plot. Compared to the PCA of the spectra, the PCA of the first derivative showed a higher correlation between tannins extracted from leathers and mimosa extract. This correlation was even higher in the PCA of the second derivative. This better performance of the derivatives, especially the second derivative, is also evident in hierarchical clustering results, where cluster plots show a decreasing gap between the extract obtained from leathers and mimosa tannins.

Therefore, considering the good performance of FTIR spectroscopy in identifying the type of tannin used in tanning, its source plant can also be determined by chemometric methods.

Conclusion

This study examined the feasibility of using FTIR spectroscopy-based chemometrics to identify condensed tannins used in the tanning of a leather product. Spectroscopy of the extracts obtained from three plant sources including mimosa, quebracho, and fruit peel of European horse-chestnut showed significant amounts of condensed tannins in these extracts. In addition to these plants, FTIR spectroscopy was performed on the extracts obtained from three specimens of roughly 6 years old mimosa tanned leathers, which were found to be rich in condensed tannins.

Principal component analysis and hierarchical clustering carried out using the obtained FTIR spectra showed that this could be a viable method to identify and classify plant species from which condensed tannins are produced. This differentiation and classification were performed through the analysis of fingerprint region spectra and their first and second derivatives. Interestingly, the multivariate analyses provided more accurate classifications when applied to the derivatives of the spectra, with the second derivative being the best

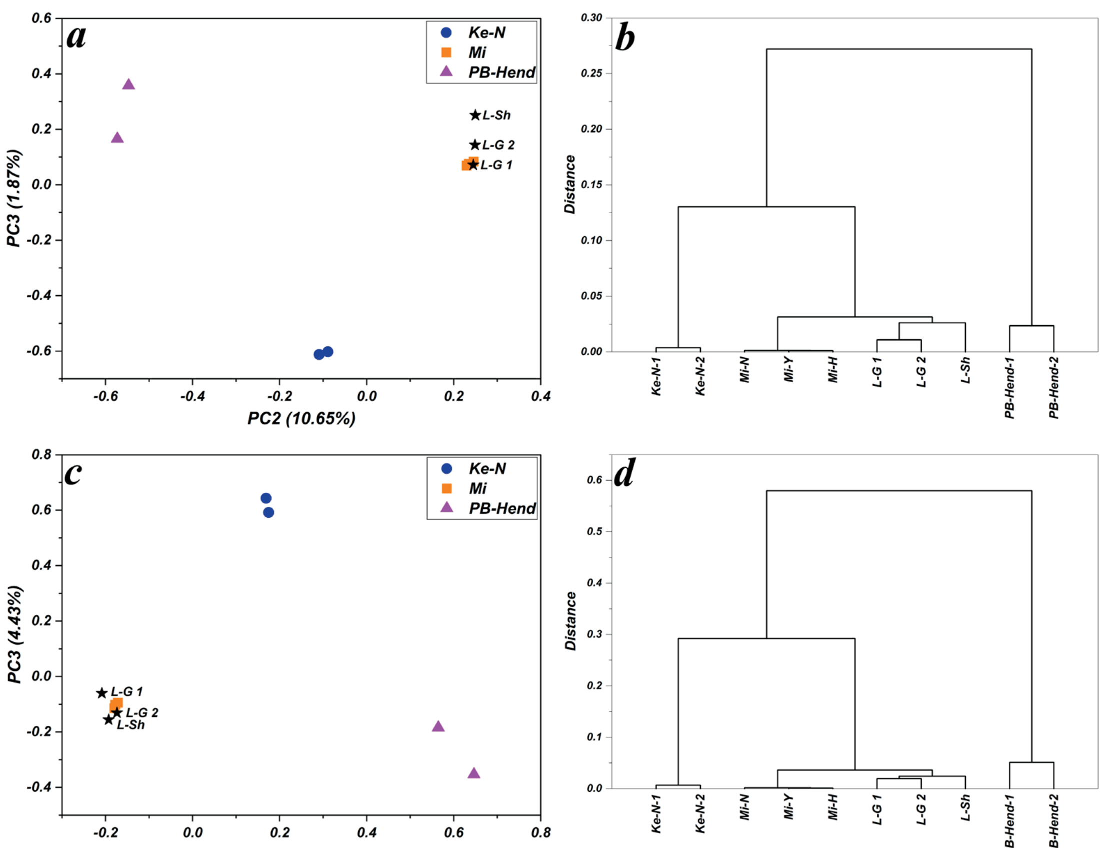

Figure 3. PCA and hierarchical clustering plots for the first derivative **(a, b)** and the second derivative **(c, d)** of the FTIR spectra of the condensed tannin extracts obtained from plants and leathers in the 700-1800cm^{-1} range

choice in this regard. The PC1-PC2 plot in the analysis of FTIR spectra and the PC2-PC3 plot in the analysis of derivatives showed the best ability to differentiate and identify the extracts. Therefore, in addition to being useful in the study of new leathers and identification of their plant sources, the combination of spectroscopic and chemometric methods can also be extended to identify the type of plants used in the tanning of historical leathers.

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