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Non-invasive and non-destructive Raman spectroscopic characterization of some Brazilian ethnographic resins

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Abstract

The chemical investigation of museum collection items can provide key information on the provenance and technical aspects involved in their production and, additionally, can help in the establishment of restoration and conservation strategies of rare or valuable assets. Natural organic materials are challenging because of their chemical complexity and expected degradation. In this work, eight ethnographic resins from different sites in the Amazon and central areas in Brazil were studied by FT-Raman spectroscopy aiming at the non-invasive and non-destructive identification of their chemical composition; chromatographic methods (Thin Layer Chromatography and Gas Chromatography–Mass Spectrometry—TLC and GC-MS, respectively) were used as destructive confirmatory techniques. The samples were classified into five groups, each one presenting a common set of spectroscopic features: diterpenoid resins with chemical composition similar to the Brazil copal in some cases with carotenoids in their composition as did the triterpenoid resins also identified. Additionally, two gums (polysaccharides) were detected, and two very dark samples only generated a thermal or a luminescent background (1064 and 632.8 nm excitation, respectively). Total ion current (TIC) chromatograms from these very dark samples only showed peaks assigned to fatty acids probably because the resinous compounds were not soluble in the solvent used in GC-MS analysis. These results show that resin-containing museum assets in the Brazilian ethnographic context can be studied directly by Raman spectroscopy, contributing to a better understand of the culture of indigenous peoples from resin provenance, uses and processing for example. The results will be used as a database to be compared with certified reference samples, when available.

KEYWORDS

Amazonian, ethnographic, GC-MS, Raman, resins

1 | INTRODUCTION

Spectroscopic techniques are extensively used in the investigation of cultural heritage objects, and among them, Raman spectroscopy occupies a leading position because of its non-invasiveness, non-destructiveness and touchless characteristics.[1] Another advantage is that Raman analysis can be performed in situ, using fibre optics to focus the laser beam and to collect the scattered light from specific spots on the object surface[2] or ex situ when sample fragments are available to be studied on a microscope stage.[3] This represents an obvious advantage over IR spectroscopy, where very rarely can a sample be studied without any type of manipulation such as solubilization in an appropriate solvent or crushing for ATR-FTIR or preparation of KBr pellets and mineral oil dispersion.

Furthermore, a Raman spectrum is much simpler to analyse than an IR spectrum, since generally, overtones and combination bands are only observed at low intensity. When the laser radiation is strongly absorbed by the sample, giving rise to a resonance Raman effect,[4] a selective enhancement of bands associated with the chromophoric group in a molecule occurs, and this is particularly convenient for the investigation of certain coloured pigments.[5]

If coupled to a microscope, another aspect that is worthy of mention is the much better spatial resolution presented by Raman microscopy (few μm^2) compared to FTIR microscopy which in the case of ATR-FTIR objective is dependent on the crystal refractive index but it is at least 1 order of magnitude larger.

All of these aspects are of special importance in the investigation of resins, since the preparation of films for FTIR analysis involves the resin solubilization in an appropriate solvent and problems with solubility can be anticipated, particularly in the case of degraded samples; partial solubility may result in selective extraction of specific fractions from the sample, which may not therefore be representative of the bulk material. Even when it is possible to collect a small amount of sample from the object, the dispersion of the resin in KBr or in a mineral oil can be difficult, giving rise to artefacts in the IR spectrum. Finally, the better spatial resolution operating at lower wavelengths (nanometres compared to micrometres) is crucial in differentiating deteriorated from non-deteriorated areas in the object[6] and in performing interfacial studies or for the identification of inclusions.

The main drawback in the application of Raman spectroscopy in cultural heritage objects is luminescence, particularly fluorescence, which is a quite common feature in organic compounds particularly when investigated with visible laser excitation. Among the possibilities available to overcome this problem, changing the exciting laser line wavelength is most commonly used. When none of the visible lines available are useful, FT-Raman instruments can be used with excitation in the near infrared (NIR) at 1064 nm, currently the longest wavelength commercially available for Raman excitation. Thus, FT-Raman spectroscopy has been largely used in the investigation of resins,[6,7] their ageing or degradation processes[7,8] and for the study of composites.[9]

In general, the study of natural resins is complicated by the fact that they present a quite complex chemical composition, are very prone to degradation and consist of a heterogeneous mixture of compounds; these factors are the main origin of the luminescence background and band broadening generally observed in the Raman spectra. Problems associated with chemical complexity and heterogeneity can be addressed by systematic investigations of resins whose chemical compositions are well known, but it has to be emphasized that the composition of a resin can change over time even considering plants or botanical sources of the same species,[10] thus highlighting the importance of combining chemical (composition and spectroscopy) with botanical information.

In the literature, much work has been focused on the identification of inorganic pigments by Raman spectroscopy[11]; however, biomaterials and resins have been substantially less investigated, probably due to the fact that sample complexity and degradation processes can make data acquisition and interpretation quite difficult.

Resins have been extensively used by many different cultures throughout history in the manufacture of decorative objects (such as small sculptures or jewellery) and as adhesive or waterproofing compounds.[12] Raman spectroscopy has been very successfully applied in the investigation of archaeological resins[7,13] including a study of an Indian lip-plug (ca. 1600 BP) found in an archaeological site in the south-east of Brazil.[14] Ethnographic resins used by different indigenous peoples in Brazil are at the focus of this investigation, aiming at the identification of the resin composition, helping to understanding their uses by comparison with data collected from objects kept in museum collections representative of Brazilian indigenous peoples. This is particularly important in the case of indigenous peoples that are disappearing, emphasizing the need for the preservation of their culture. In a more general perspective, resins from South America are not as investigated as those from Africa[15] and Asia[16]; thus, the construction of a database is mandatory for artefacts from this source and will permit future comparisons with the data available in the literature from geographically different sites and cultures.

In this work, eight ethnographic resins from different sites in the Amazon and central areas in Brazil were analysed by FT-Raman spectroscopy for the non-invasive

and non-destructive identification of their chemical composition. Destructive chromatographic techniques (TLC and GC-MS) were used to provide confirmation of the FT-Raman spectroscopy findings.

2 | EXPERIMENTAL

The ethnographic resins comprised eight different samples, here identified in roman numerals from I to VIII, which belong to the Museum of Archaeology and Ethnology of the University of São Paulo (MAE-USP). They were collected from the Amazon and Central areas in Brazil, and their ages range from about 40 to 100 years BP (Table 1). Externally, they generally present yellow (I, III, IV and VIII), red (II), dark brown (V) or black colours (VI and VII). An inspection of the internal regions of the black and dark brown samples frequently reveals brighter coloured and, in most cases, red resins, as in the case of resins I, II and V. A detailed description of the samples is given in Table 1. Brazilian copal and dammar were used as reference standard examples of diterpenoid and triterpenoid resins, respectively, and are not included in Table 1. Dammar (solid resin) was purchased from a local supplier (Casa do Restaurador, São Paulo, Brazil), and the Brazilian copal was a reference sample provided by Dr. Luiz Antonio C. Souza (Federal University of Minas Gerais, Minas Gerais, Brazil).

For the Raman analysis, no sample preparation was required, and the resins were studied as received; when possible, visibly non-degraded areas were chosen as they provide higher quality spectra and more representative data. For the TLC and GC-MS experiments, all chemicals were analytical grade (Merck, Argentina).

TLC analysis was performed on pre-coated Si gel F254 (Merck) using benzene: MeOH (95:5) and the spots were detected by spraying with $SbCl_3$ 20% in $CHCl_3$.[17] In the case of the TLC monosaccharide analysis, the mobile phase was CH_3CN:H_2O (85:15), and the spraying reagent was a solution of 5% *p*-anisaldehyde in ethanol: H_2SO_4:glacial acetic acid (36:2:0.1). For the TLC carotenoid analysis, the resins were dissolved in ethyl acetate, and a *t*-butanol:cyclohexane (2:8) mixture was used as the mobile phase; H_2SO_4 10% was employed as a detection reagent.[18]

Acid hydrolysis of samples IV and VIII (1 mg) was carried out heating the resins in a Pierce vial with HCl 0.3 mol/L (0.5 ml) at 95°C for 5 h. After evaporation under nitrogen, the residues were dissolved in methanol and analysed by TLC.[17] For the saponification and methylation of the other resins, samples (10 mg) were extracted in CH_2Cl_2 (I–V and dammar) or ethyl acetate (VI, VII, and Brazil copal) (2 ml) by sonication at room temperature. After centrifugation, aliquots of the supernatant organic extracts were evaporated under nitrogen and subjected to saponification with KOH 4% in methanol for 1 h at 70°C. Neutral organic components were extracted with cyclohexane. After acidification to pH = 1 with HCl 2 mol/L, the acidic organic components were extracted from the residual solution with diethyl ether,

TABLE 1 Description of ethnographic resins studied in this work

Sample	Collection number	Cultural group	Geographic origin	Year collected	Information available	Notes
I	9524	Kanamari (Tukuna)	Amazon State	1958	'Red resin for ceramics'	Externally yellow and internally red-orange
II	5053	Itya Mahãdu (Javaé)	Goiás State	1909	-	Externally black and internally Bright red
III	5050	Itya Mahãdu (Javaé)	-	-	-	-
IV	2816	Iny (Karajá)?	-	-	-	-
V	6952	Huni kuin (Kaxinawá)	Curanja river at the Peruvian border	1950	-	Sample wrapped in leaves. Externally black and internally red, soft and sticky
VI	6956	Huni kuin (Kaxinawá)	-	-	-	Resin rod. Externally black and rigid and internally sticky
VII	12 318	Ka'apor (Urubu)	Maranhão State	1966	'Resin for restoration'	Externally and internally dark brown and hard
VIII	114	Mehin (Krahô)	Goiás State	1944	'Angico resin'	Glassy shine inside

Note: Their photographs are available in Figure S1. All the samples belong to the Museum of Archaeology and Ethnology of the University of São Paulo, São Paulo, Brazil.

followed by evaporation under nitrogen. Methyl esters were obtained by derivatization with BF_3-methanol 20% at 100°C for 3 min, extraction with $CHCl_3$:H_2O (1:1) and evaporation under nitrogen for further analysis by GC-MS.[19]

FT-Raman spectra were obtained using a Bruker RFS-100/S fitted with a liquid N_2 cooled Ge detector and with a Nd^{3+}/YAG laser (1064 nm); the laser spot was approximately 100 μm in diameter at the sample. Spectra were recorded with 4 cm^{-1} spectral resolution, and the number of scans accumulated to provide better signal-to-noise ratio was dependent on the specific sample. At least nine spectra were collected for each sample, and laser power at the sample was 70 mW which proved to be safe for the resins, including the darker ones. Raman spectra excited in the visible were recorded using a Renishaw Raman Microscope System 3000, using the 632.8 nm line of a He-Ne laser (Spectra Physics, mod. 127). All the spectra were processed using Grams/AI spectroscopy software package.

GC-MS was performed on a TRIO-2 VG mass spectrometer (electron impact 70 eV, ion source temperature 180°C, interface temperature 250°C) coupled to a Hewlett-Packard 5890 chromatograph equipped with a flame ionization detector and an ULTRA 1 column (25 m × 0.20 mm i.d.). The temperature programme was 2 min of isothermal at 80°C and then 80 to 290°C at 8°C min^{-1}, followed by a 25 min hold at 290°C. Helium was used as the carrier gas and inlet pressure was 7 psi.

3 | RESULTS

The emphasis of this work is the use of Raman spectroscopy in the direct characterization of resins with historical and/or cultural relevance, since it is a non-destructive technique, providing chemical information that can be valuable in the ethnographic context as well as for provenance, conservation and restoration purposes. Other techniques, including FTIR and GC-MS, are also largely used in the identification of organic materials in the museum context, but in these cases, some small amount of material must be destroyed or manipulated in the course of the analysis. Nevertheless, these techniques provide very useful chemical and structural information. TLC and GC-MS were used here to validate the initial identification made by Raman spectroscopy.

Excitation in the visible region of the electromagnetic spectrum was also used, but the data quality is much poorer than with excitation in the NIR due to the luminescence background, and, for this reason, in this work, only the FT-Raman results are reported. One drawback experienced in FT-Raman spectroscopy is the thermal emission (black body radiation) generated by black or very dark coloured samples that absorb a fraction of the incident laser beam; black body emission results from the energy relaxation that follows the absorption.[20] Consequently, some samples are very challenging for Raman spectroscopy as they present a luminescent emission background when excited in the visible or thermal background when excited at 1064 nm. This is the case found for samples VI and VII; resin VIII was also difficult to analyse due to the background emission intensity, but it was possible to identify some bands after baseline correction (not shown), although with a very poor signal/noise ratio.

In Figure 1, the Raman spectra of resins I–V are presented; as mentioned above, resins VI and VII generated only a broad emission background and were not included in the figure, whereas the spectrum of resin VIII is similar to resin IV and will be shown elsewhere.

It is clear from Figure 1 that although there are some similarities, the spectrum of resin III is different from resins I, II and V, as the main peaks at ~1155 and

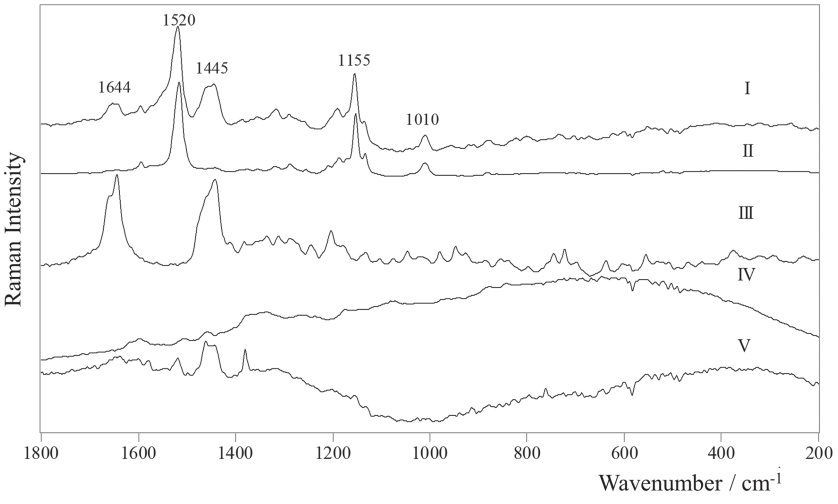

FIGURE 1 FT-Raman spectra of ethnographic resins I–V

~1520 cm^{-1} are absent; these two strong bands indicate that carotenoids are present,[21] and, as will be discussed later, this was confirmed by TLC. It can be seen in Figure 2 that the most significant bands in the resin III spectrum are exhibited at 724, 1180, 1204, 1445, 1460, 1644 and 1662 (shoulder), and a comparison with the spectrum of a resin that is known to be rich in diterpenoids (*Hymaneae Stig. Mart.*, Brazil copal) suggests that sample III can be described as a diterpenoid resin; bands at 2870, 2930 and 3080 cm^{-1} are also observed but are not shown in the figure. The same happens with resin II after expanding the weaker bands: the main features at 1010, 1135, 1189, 1213, 1443 and 1642 cm^{-1} are in agreement with a resin with structure is mostly based in the labdane diterpenoid group.[5]

In the case of resins I and V, the carotenoid signals are also clearly observed, but the other bands does not match with the previously considered diterpenoid resin. Mostly because of the spectral profile in the 1600–1700 cm^{-1} region, resins I and V present features that are suggestive of triterpenoids,[5] as previously observed in the case of a lip plug (*tembetá*) investigation[14] and when the dammar spectrum is used for comparison a good match of the main features is achieved (Figure 3).

A completely different behaviour was observed for samples IV and VIII as can be seen in Figure 4. The bands are broad, the S/N ratio is poor and the spectral features do not fit into the two previously discussed patterns, with the most significant bands being observed at 844, 880, 1085, 1260, 1350, 1460 and 1600 cm^{-1}. According to a Raman spectroscopic protocol for resin differentiation available in the literature,[5] bands of similar intensities at ~800 and 1650 cm^{-1}, as observed for these two resins, are indicative of polysaccharides (gums).

From the spectral pattern observed in the Raman spectra for all the eight samples, the resins can be arranged into five classes: (i) triterpenoid (dammar type) containing carotenoid compounds (identified by the characteristic strong peaks at ~1155 and 1520 cm^{-1}), represented by resins I and V; (ii) diterpenoid (Brazil copal type) with carotenoids (resin II); (iii) diterpenic with chemical composition similar to the Brazil copal resin (resin III); (iv) gums (bands of similar intensity at 800 and 1650 cm^{-1}) represented by samples IV and VIII

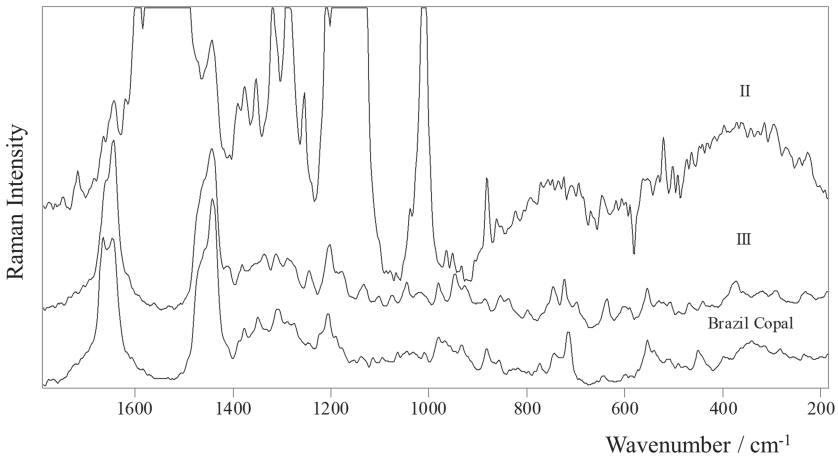

FIGURE 2 FT-Raman spectrum of resins II and III and Brazil copal

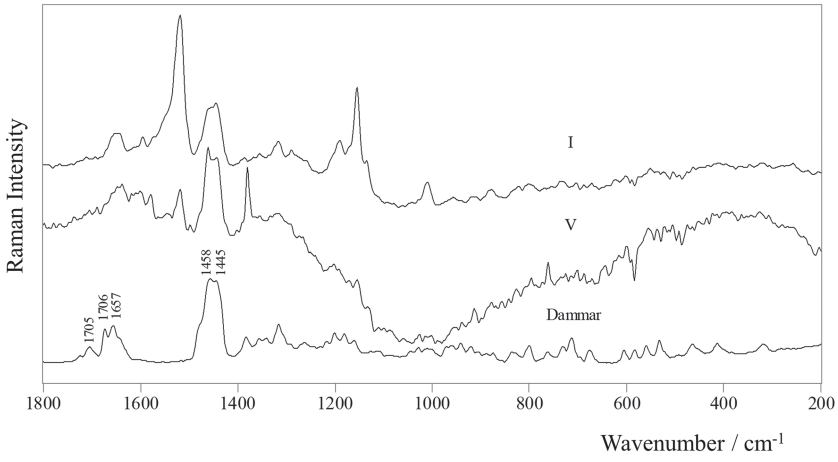

FIGURE 3 FT-Raman spectrum of resins I and V and dammar

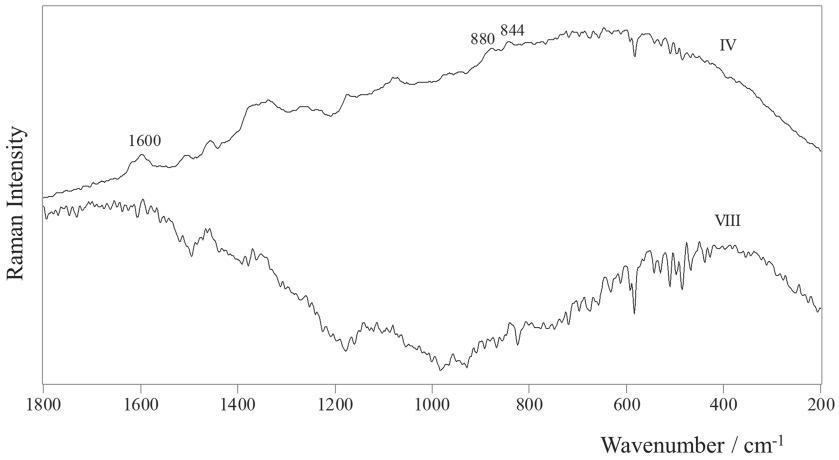

FIGURE 4 FT-Raman spectrum of resins IV and VIII

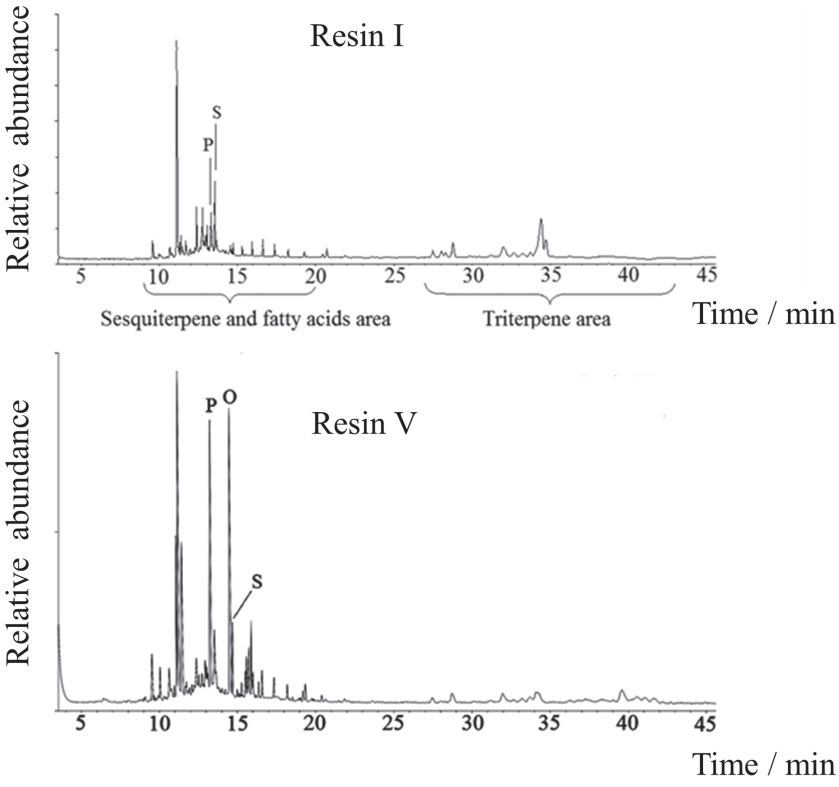

FIGURE 5 Total ion current (TIC) chromatogram of resins I (top) and V (bottom). P, methyl palmitate; S, methyl stearate; O, methyl oleate

and (v) black samples, which were not possible to investigate by Raman spectroscopy as the thermal or fluorescent background swamped the vibrational bands, as observed in the case of resins VI and VII.

This Raman-based classification was subjected to a comparison using TLC and GC-MS as chosen confirmatory techniques, and the results are summarized here. The resins were tested for solubility in ethyl acetate and CH_2Cl_2. In a polar solvent such as ethyl acetate, samples II, IV and VIII were fully soluble, whereas an insoluble residue remained in the case of resins I, III, V, VI and VII. TLC was performed for the ethyl acetate solutions of the eight samples and for the hydrolysis products of samples IV and VIII, as described in Section 2. It was observed that the chromatographic patterns of samples I and V resemble that of dammar resin while those of samples II and III are similar to a Brazil copal resin. The pattern of spots for the hydrolysis products of samples IV and VIII is characteristic of carbohydrates, suggesting that these samples are gums. TLC analysis of the ethyl acetate extracts of samples I, II and V showed spots characteristic of carotenoids, confirming the presence of this type of compounds in the resins.

GC-MS was employed to characterize the composition of the acidic fraction of samples I–VII (resins IV and VIII were previously assigned to carbohydrates). The total ion current (TIC) chromatograms of samples I and V (Figure 5) exhibit groups of fatty acid compounds and

sesquiterpenes, eluting between 9 and 20 min, and of triterpenoids, eluting after 27 min. Palmitic (16:0) and stearic (18:0) acids are the main fatty acids in both resins, with a high amount of oleic (18:1) acid in resin V. Mass spectral study of the major compounds of the triterpenic fraction of both resins shows peaks at *m/z* 262, 203 and 189 characteristic of oleane or ursane related triterpenoids with an aldehyde or carbomethoxy group at C-17 or C-28 (not shown).[22,23]

The TIC chromatograms of resins II and III (Figure 6) show a group of diterpenoids eluting between 15 and 20 min. In the case of resin II, a minor fraction of fatty acid compounds (palmitic (16:0), stearic (18:0) and oleic (18:1) acids) eluting between 13 and 15 min is also observed. The mass spectra of the major diterpenic compounds in both resins exhibit a peak at *m/z* 191 (not shown), characteristic of bicyclic diterpenoids of the labdane group, similar to those of Brazilian copal.[23,24]

TIC chromatograms from samples VI and VII (not shown) have peaks assigned to branched and linear fatty acids, respectively, and no resinous compounds were detected in both cases. This fact can be explained considering that such samples are very badly degraded and only partially soluble in ethyl acetate; thus, it is very likely that the highly polymerized resin remained in the solid residue and the CG-MS analysis only reflects the fatty acid (soluble) fraction composition.

4 | DISCUSSION

Previous research on a discrimination protocol for diterpenoids and triterpenoids has shown that the

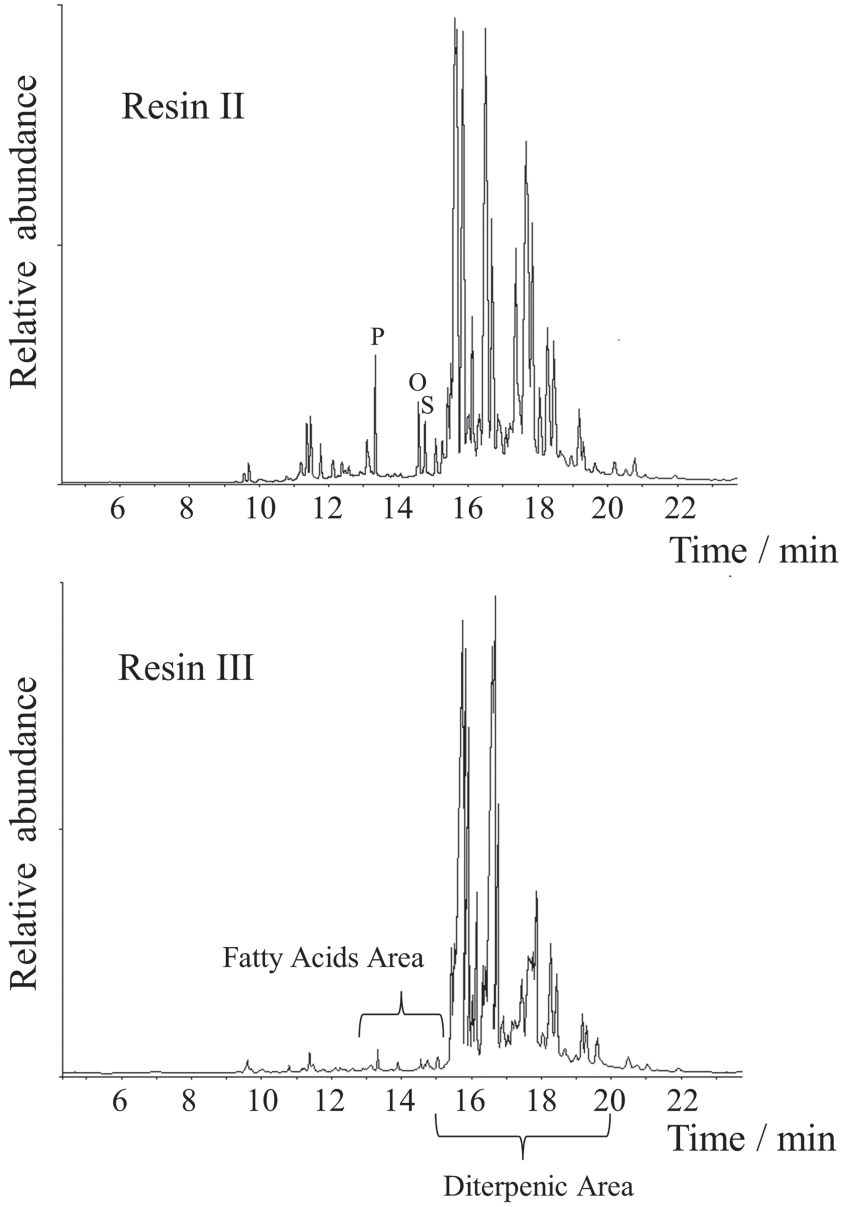

FIGURE 6 Total ion current (TIC) chromatogram of resins II (top) and III (bottom). P, methyl palmitate; S, methyl stearate; O, methyl oleate

wavenumber region 1520–1750 cm^{-1} is crucial for terpenoids speciation.[5,13] Such a protocol was employed in a previous study aiming at the characterization of an archaeological resin used in the manufacture of an Indian lip-plug (*tembetá*)[14]: Where the Raman spectra of the archaeological *tembetá* was compared with *Pinus halepensis*, *Cedrus libani* (both diterpenoid) and *Pistacia lentiscus* (triterpenic) resins, the presence of two major Raman bands in this region (at 1656 and 1702 cm^{-1}) was the main spectroscopic reason used to ascribe it as a triterpenoid resin. This procedure was based on the observation that diterpenoid resins, containing predominantly abietane compounds, have strong bands at approximately 1650 (or lower) and 1611 cm^{-1}, sometimes with a band near 1635 cm^{-1} whose intensity increases with specimen degradation. On the other hand, *Pistacia* resins always have a slightly higher wavenumber feature at about 1655 cm^{-1} and a weaker band near 1705 cm^{-1}; degradation produces a weaker shoulder around 1620 cm^{-1}. The presence of a doublet at about 1460/1440 cm^{-1}, with the two bands having similar intensity, confirmed the triterpenoid chemical nature of the *tembetá* resin.[5]

The successful investigation of the *tembetá* inspired the current investigation of ethnographic resins collected decades ago mostly in the Amazonian region. The precise geographical location of such indigenous peoples is not in the museum records (see Table 1), but some of them were frequently moving populations till the creation of the Xingu Indigenous Park in 1961[25] making their location troublesome; without such information, it is very difficult to identify of the botanical origin of the resins as certified samples for comparison are necessary. Furthermore, there is almost no information available for the resins (resin I, 'red resin for ceramics'; resin VII, 'resin for restoration'; resin VIII, 'angico resin') making impossible the association between chemical composition and application. However, Raman spectroscopy proved to be an extremely useful non-destructive tool in resins identification[5] and therefore was used in this work to characterize the ethnographic resins listed in Table 1 aiming at the construction of a database which can be applied to analyse resin-containing museum objects. As mentioned before, the chemical composition of a resin can be important from the ethnographic point of view, for provenance identification and conservation and restoration purposes.

Thus, the same protocol used with the tembetá was employed to characterize the ethnographic resins here studied and a comparison with a diterpenoid (*Hymenaea stigonocarpa Mart.*, Brazil copal) and with a triterpenoid resin (dammar) was made. As expected, the Brazil copal resin (diterpenoid) showed a strong feature at 1634 cm^{-1}, with weaker shoulders at about 1650 and 1640 cm^{-1} whereas the spectrum of dammar (triterpenoid) presented bands at 1657, 1673, 1705 and 1725 cm^{-1}; a doublet at 1445 and 1459 cm^{-1} was not observed directly due to band broadening, but it is clearly disclosed after resolution enhancement procedure (Fourier Self Deconvolution, not shown).

The results obtained have revealed some interesting features: Although the resins look similar by visual inspection, their Raman spectra are significantly different allowing the samples to be classified into five groups, as presented in Section 3.

The only information which could lead to the identification of one of the resins was available for sample VIII ('Angico resin'). Angico is a *Fabaceae* tree, found from the Amazon to the southern States such as Paraná and São Paulo States; white angico (*Anadenanthera colubrina*) and red angico (*Anadenanthera macrocarpa [Benth]*) produce an exudate with similar chemical composition, rich in arabinose (~65%) and galactose (~25%).[26,27] Therefore, although Raman spectroscopy was not able to identify the carbohydrates present in the samples, it correctly detected their chemical nature as gums. The number of applications described for the angico polysaccharides is large, including healing and adhesive properties, but as with resin IV, it certainly was not used in pottery waterproofing because of its water solubility.

Natural resins undergo significant oxidation and polymerization reactions due to natural ageing, resulting in mixtures of oxidized compounds not present in the fresh resins; therefore, it is necessary to use a confirmatory technique to ensure that the Raman data are not affected by contaminations or degradation products. GC-MS is one of the most widely used techniques for the analysis of complex mixtures of organic compounds and together with TLC has been applied successfully to the identification of lipids and resins in ethnographic and archaeological samples[28,29]; GC-MS analysis of resins I–V allowed us to identify the presence of oleane or ursane type triterpenoids in resins I and V, while resins II and III are composed of labdane diterpenoids. The triterpenoid composition of resins I and V is characteristic of dammar and mastic resins while *Hymenaea* species belonging to resin-producing trees of the family *Leguminosae* is rich in labdane diterpenoids.[30] In some resins (I, II and V), carotenoids (possibly β-carotene considering the band positions in the Raman spectrum)[9] were detected which, at least in part, can be associated with their reddish colour. Carotenoids, a class of mostly C40-based lipophilic tetraterpenes,[31] play an enormous number of roles in plants, animals and humans,[32] but as far as the authors are aware, the presence of carotenoids in resins is not usual; although exudates are still used as medicine by local inhabitants[33] and carotenoids are known for their antioxidant properties,[31] it is not possible to

ascribe a pharmacological use to the resins-containing carotenoids here studied based on the information reported.

As mentioned before, the protocol used in the Raman investigation of the ethnographic resins here characterized is based on previous studies reported in the literature involving a large number of resins and gums from different sources; unfortunately, none of them included resins from South America, particularly from the Amazon region, to allow a direct comparison. In the ethnographic context, even less information is available[34] especially when there are no documented uses associated to the resin.

5 | CONCLUSIONS

Raman spectroscopy was successfully used in the investigation of ethnographic resins aiming to obtain information on its chemical composition, in a non-invasive and non-destructive procedure. Excitation in the NIR was used due to the fluorescence presented by most samples, and good quality spectra were obtained for all the resins except the very dark ones, in which case the heating caused by the laser line (1064 nm) absorption creates a thermal background that swamps the Raman bands, whereas in the visible (632.8 nm), the fluorescence background hampered the observation of the vibrational bands. Raman data were confirmed by TLC and GC-MS.

The results here reported are the first step towards the non-invasive identification of Brazilian ethnographic resins used in museum assets, therefore enlarging the knowledge of culture of indigenous peoples, currently threatened by illegal loggers, miners and ranchers. Furthermore, the resins identification will contribute in the choice of restoration procedures and preservation strategies to be applied to such assets.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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