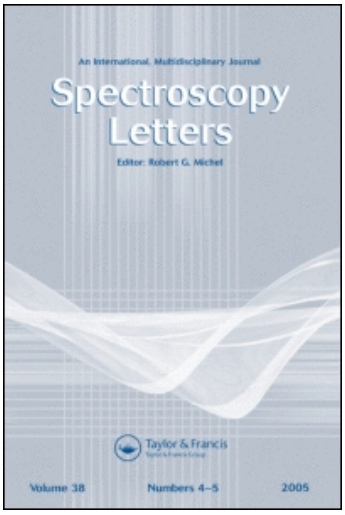

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Raman Spectroscopic Characterization of the Alkaloid Dihydrochelerytrine Extracted from Roots of *Zanthoxylum stelligerum* (Turcz)

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ABSTRACT Raman spectroscopy has been used successfully in the identification of the alkaloid dihydrochelerytrine due to the assignment of specific key marker bands in the region between 1000 and 1600 cm^{-1}. The Raman spectrum obtained from the crude hexane extract of the roots of *Zanthoxylum stelligerum*, and excited with 1064 nm, provides very good molecular information, as can be seen by the comparison between the Raman spectra of the standard dihydrochelerytrine and the crude extract, where the keymarker bands are present in both spectra.

KEYWORDS alkaloid, dihydrochelerytrine, FT-Raman, *Zanthoxylum stelligerum*

INTRODUCTION

The biological activity of metabolites extracted from plants has always been a critical parameter for prospecting or for the production of new drugs, as well as for the study of the biochemistry of their operation. The appearance of multiresistant strains of microorganisms to the known antimicrobial drugs has led to the search for new and more powerful antibiotics. Therefore, metabolites derived from plants, particularly those from regional flora, can be a viable and reliable research activity in the search for new prototype drugs.[1]

The Rutaceae family is a well-known producer of alkaloids, coumarins, mono- and sesquiterpenes, as well as phenylpropanoids;[2–4] these metabolites possess a wide range of biological activities, of which one of the most important is antibiotic activity,[5] but have also been studied *in vitro* and *in vivo* in several different uses, as for instance as anticancer agents,[3,6] against *Trypanosoma cruzi*,[7] as well as for anti-inflammatory and analgesic activities.[8] Among the Rutaceae family, *Sanguinarina canadensis* Linné (Papaveraceae) contains sanguinarin, a benzophenatridine alkaloid derivative with emetic and anti-inflammatory properties that can be extracted from the roots of the plant and can be incorporated in toothpastes and oral dental

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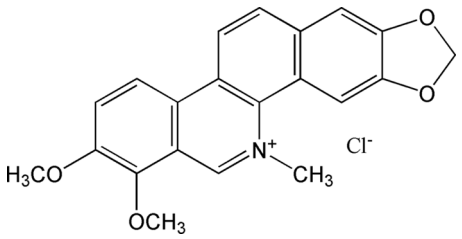

FIGURE 1 **Molecular formula of dihydrochelerytrine.**

washes to prevent periodonsis,[9] as it has a wide antimicrobial action and is inhibitory to bacterial adherence.

Substances with very similar chemical structures to sanguinarin can be found from different species of *Zanthoxylum* (Rutaceae),[10–13] some of them occurring in the semiarid region of Bahia State in the northeast of Brazil. More specifically, in this current work, specimens of *Zanthoxylum stelligerum* (Turcz) were collected and the root extracts investigated by means of Raman spectroscopy, using near-infrared laser excitation (1064 nm). Raman spectroscopy has become one of the most important nondestructive methods to evaluate the qualitative and quantitative presence of several different chemicals in plants[14–17] in a search for an efficient measurement of valuable biochemical plant components. It is noteworthy that *Zanthoxylum stelligerum* (Turcz) has not been investigated by means of vibrational spectroscopic methods, nor has the most important chemical present in the roots of *Zanthoxylum stelligerum*, the alkaloid dihydrochelerytrine, whose structure can be seen in Fig. 1. In this sense, this work deals with the description of the most important vibrational bands of dihydrochelerytrine, in order to determine the keymarker bands, and of the extract from *Zanthoxylum stelligeru,* proposing it as a rapid and efficient methodology to identify the plant and the chemical composition of the extract, which can be very important for the pharmaceutical and phytotherapeutic industries.

MATERIALS AND METHODS

Specimens of *Zanthoxylum stelligerum* were collected at Mucuge City, Chapada Diamantina, Bahia State, in northeast Brazil. After identification, a standard archival specimen was deposited at the Institute of Biology at Universidade Federal da Bahia (Salvador, Bahia State, Brazil).

The extract was prepared as follows: ground roots of *Z. stelligerum* were extracted through maceration with hexane. The purification and isolation of dihydrochelerytrine was accomplished by conventional phytochemical procedures, including thin-layer and column chromatography and recrystallization in ethanol. Low-resolution mass spectroscopy provided a peak related to an M+ at m/z 349 (100%), which is compatible with a $C_{21}H_{19}O_4N$ molecular formula. Carbon and hydrogen NMR spectra were used to confirm the structure of dihydrochelerytrine as the most abundant alkaloid component present in the roots of *Z. stelligerum*. Dihydrochelerytrine was used as a standard from Aldrich without further treatment.

Fourier-transform Raman spectra were obtained using a Bruker IFS66/FRA 106 instrument with a Nd^{3+}/YAG laser operating at 1064 nm with a 4 cm^{-1} spectral resolution and 500 spectral scans accumulated to improve the signal-to-noise ratio; laser powers were maintained at 50 mW or less at the samples to prevent possible damage to the biological materials. Wavenumbers of strong/sharp bands are accurate to ± 1 cm^{-1} or better, and all spectra were recorded in triplicate for all samples to demonstrate that no thermal or photodecomposition had occurred during the spectroscopic analyses.

RESULTS AND DISCUSSION

In the low-resolution mass spectrum obtained for the extract from *Zanthoxylum stelligerum*, the parent peak related to M+ in m/z 349 (100%) can be observed, compatible with a $C_{21}H_{19}O_4N$ molecular formula. In the ^{1}H NMR spectrum can be observed, in the aromatic region, the presence of 6 signals corresponding with two singlets (each integrated for one hydrogen) and four doublets (integrated for 4 hydrogens). The coupling constants of these signals ($J = 8.7$ Hz) indicate that these atoms are located in an ortho position relative to each other. The analysis of the ^{1}H NMR spectrum also reveals the presence of a methylenedioxy group (due to a singlet at 6.05 δ) and a methoxy group at 3.89 (s, 3H) and 3.93 δ (s, 3H). The summation of the signal integration shows that this molecule has 19 hydrogen atoms. The ^{13}C NMR spectrum indicates the presence of 21 carbon atoms in the structure, 6 of these being aromatic in character. All these data are in good

TABLE 1 **Main Wavenumbers (in cm^{-1}) and Tentative Assignments for Dihydrochelerytrine and the Hexane Extract Obtained from *Zanthoxylum stelligerum***

Dihydrochelerytrine	*Zanthoxylum stelligerum* extract	Tentative assignment
1640 w	1643 m	ν(C=N) + ν(C=C)
1602 s	1603 vs	ν(C=C) + ν(C=N)
1570 m	1574 m	ν(C=C) + ν(C=N)
1521 m	1523 m	ν(C=C) + ν(C=N)
1461 m	1461 s	CH deformation
	1443 ms	CH in-plane bend
1359 s	1358 s	CH in-plane bend
1340 wm		CH in-plane bend
1273 wm	1276 w	CC and CNC in-plane bend
1085 v w		Skeletal stretch (dioxolene)
1040 v w		Skeletal stretch (dioxolene)
709 wm	707 w	Ring deformation (dioxolene)

Abbreviations for intensity: vw, very weak; w, weak; wm, weak to medium; m, medium; s, strong.

agreement with the dihydrochelerytrine structure reported by Facundo et al.[18]

The tentative assignment of the vibrational bands, which is presented in Table 1, was made based on the mode descriptions of similar molecules.[19–23] The infrared spectrum of the extract obtained from *Zanthoxylum stelligerum* shows absorption bands at 1464, 1494, 1600, and 3004 cm^{-1}, which can be assigned to C−H in-plane bending, C=C stretching, and aromatic C−H stretching, respectively. The bands at 1040 and 1334 cm^{-1} can be ascribed to C−O, O−C, and C−N bonds, respectively, and can be tentatively attributed to bands that are markers in the infrared spectrum of dihydrochelerytrine. However, the presence of a relatively large number of bands in the infrared spectrum is found to be a common complication in the detailed molecular analysis of the individual substances present in plant extracts, as is seen to be the case in the current investigation.

The Raman spectrum of dihydrochelerytrine can be seen in Fig. 2, and some of the main vibrational bands are displayed in Table 1. The most intense bands are the ones at 1602 and 1359 cm^{-1}, which can be assigned to ν(C=C) + ν(C=N) stretching and CH in-plane bending, respectively. The first Raman band (1602 cm^{-1}) can also be used as a key biomarker of dihydrochelerytrine, as the other intense band refers to the CH bending mode, which is very commonly found at or near this wavenumber in organic compounds. Another vibrational band that could be used as a key biomarker would be that at 1040 cm^{-1}, assigned to a skeletal stretch involving the C–O bond; however, this band appears as a very weak feature in the Raman spectrum of dihydrochelerytrine and cannot be seen in the spectrum of the extract obtained from *Zanthoxylum stelligerum,* which will be discussed further below. The bands in the 1500–1600 cm^{-1} region can all be assigned to ν(C=C) + ν(C=N) coupled modes. The band at 1085 cm^{-1}, which is tentatively assigned to a skeletal stretching motion involving vibration of the ether linkage (the same vibration that also gives rise to the 1040 cm^{-1} mode), is also very characteristic of a furan-like species;[22,23] however, the very low intensity of such a mode negates its assignment as a key biomarker for dihydrochelerytrine. The other bands in Table 1 are all tentatively assigned on the basis of other molecules, but none of these can be considered specific to the chemical structure investigated here.

The hexane extract of *Zanthoxylum stelligerum* was also analyzed, and the Raman spectrum can be seen in Fig. 3; the main vibrational bands are presented in Table 1. As can be very clearly seen in Table 1, the main bands present in the Raman spectrum of our standard (dihydrochelerytrine) are also

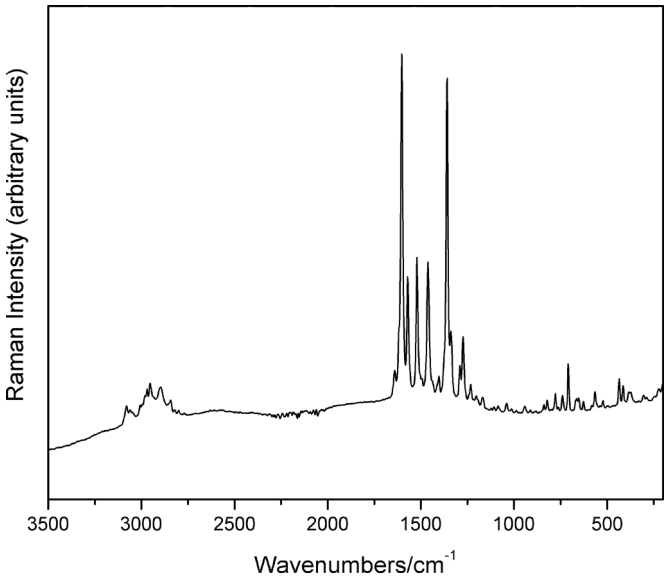

FIGURE 2 **FT-Raman spectrum of solid dihydrochelerytrine (standard). Spectrum conditions are described in the ''Materials and Methods'' section.**

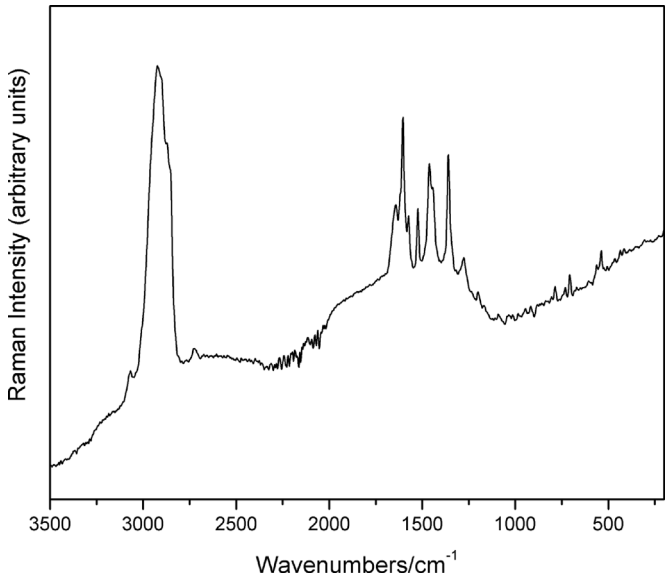

FIGURE 3 **FT-Raman spectrum of *Zanthoxylum stelligerum* extract. Spectrum conditions are described in the "Materials and Methods" section.**

displayed in the spectrum of the extract. This is a very strong indication that in the extract, at least the main component identifiable from the Raman spectrum is dihydrochelerytrine. Figure 4 shows the Raman spectra of both dihydrochelerytrine and the extract in the region between 1800 and 1000 cm^{-1}. The bands at 1462, 1519, 1570, and 1603 cm^{-1} are present in both spectra, and they relate to dihydrochelerytrine. Other bands present in the extract spectrum, for instance those at 1443 and 1643 cm^{-1} (with a shoulder at 1620 cm^{-1}), are probably due to other compounds extracted along with the alkaloid. The band at 1462 cm^{-1} is assignable to a CH_2 deformation, whereas the band at 1643 cm^{-1} is characteristic of a C=C and/or a carbonyl group; this last feature is not present in the structure of dihydrochelerytrine but may be present in other chemicals present in the raw extract. The literature has shown only a few examples of Raman spectra of alkaloids obtained from plants; for example, piperine from pepper,[24] poppy plant material,[25] theobromine from guarana,[14] among others.[1] Despite the different types of chemical structures in those alkaloids, some of the key markers found here for dihydrochelerytrine can also be applied to other alkaloids.[1]

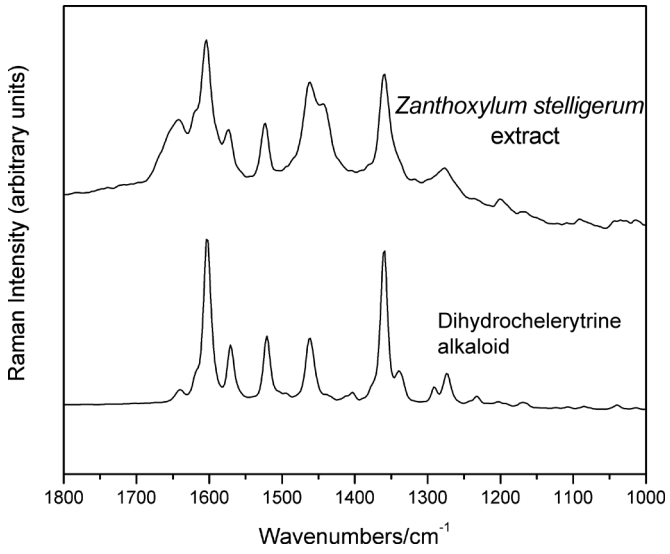

FIGURE 4 **Comparison between the FT-Raman spectra of dihydrochelerytrine (standard) and *Zanthoxylum stelligerum* extract in the 1800–1000 cm^{-1} region.**

In summary, Raman spectroscopy can be used successfully in the identification of dihydrochelerytrine due to the assignment of specific key spectral marker bands in the region between 1000 and 1600 cm^{-1}. The Raman spectrum obtained from the crude hexane extract, and excited with a 1064-nm laser, provides very good molecular information, as can be seen by the comparison between the Raman spectra of the standard dihydrochelerytrine and the crude extract obtained from the roots of *Zanthoxylum stelligerum*.

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