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Application of analytical methods for the structural characterization and purity assessment of *N*,*N*-dimethyltryptamine, a potent psychedelic agent isolated from *Mimosa tenuiflora* inner barks

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ABSTRACT

N,*N*-dimethyltryptamine (DMT) is a psychoactive indole alkaloid present in beverages consumed in religious ceremonies and in neo-shamanic rituals all around the world. It is a substance banned in most countries, which makes its acquisition difficult. In Brazil, a beverage rich in DMT named ayahuasca is legally consumed in a religious context. On the other hand, DMT is a controlled drug, enforced by the Brazilian National Health Surveillance Agency (*Agência Nacional de Vigilância Sanitária*). The present study describes a simple and fast method to obtain *N*,*N*-dimethyltryptamine (DMT) from inner barks of *Mimosa tenuiflora* for the purpose of using it as a chromatographic analytical standard. Fourier transform infrared spectroscopy (FTIR), single and tandem stage mass spectrometry (MS), nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) and melting point measurements were performed for the structural characterization of *N*,*N*-dimethyltryptamine. The results obtained were in agreement with previous literature reports. The purity of the compound (>95%) was determined using ultraviolet (UV) absorption spectrometry with a tryptamine analytical standard.

1. Introduction

Tryptamine derivatives are simple indole alkaloids widely present in biota. *N*,*N*-dimethyltryptamine (DMT) (Fig. 1) was first synthesized in 1931, and was isolated from *Mimosa tenuiflora* by Oswaldo Gonçalves de Lima in 1942 [1]. Its hallucinogenic properties were confirmed in 1956. It can be found in a wide range of plants, as well as in the human body [2,3].

Species of the Mimosoideae, a botanical subfamily of the Fabaceae family, are found in northeast Brazil, where some of these plants are known as "jurema". *M. tenuiflora* (Willd.) Poiret or "jurema-preta" (black jurema) is used as the main ingredient in "vinho da jurema" (jurema wine), since its inner barks of stems and roots are rich in DMT. The drink has indigenous origins, and is used in the rituals of several religious groups and neo-shamanic cults, due to the intermingling of Amerindian, African and European cultures [4,5]. Jurema wine, made from the inner bark of *M. tenuiflora* with addition of *Peganum harmala* seeds, contains DMT and MAOi (monoamine oxidase enzyme inhibitors), the same active principles present in ayahuasca, which is also an indigenous psychoactive beverage used in syncretic religions worldwide [6]. In Brazil, ayahuasca is legal if consumed during the course of religious activities, even by children and pregnant women [7].

Despite being present in the human body, DMT has been classified internationally as a Schedule 1 controlled drug, following the 1971 United Nations Convention on Psychotropic Substances [8]. However, consideration needs to be given to the therapeutic potential of psychedelic drugs. This is especially important because research on this matter was interrupted in the late 1960s [9–11]. The first subsequent work was conducted by Dr. Strassman between 1990 and 1995, in a clinical research approved by the US DEA (United States Drug Enforcement Administration), during which around four hundred doses of DMT were administered to sixty volunteers [12]. There is currently increased interest in the mode of action of DMT in the brain [8,13–18].

Studies involving the determination of this tryptamine compound in plant matrices, as well as in ritual beverages, are essential given the current expansion in its use for religious and recreational purposes [4,19]. Several methodologies have recently been developed to quantify DMT in plants, as well as in the beverages used in religious practices [4],

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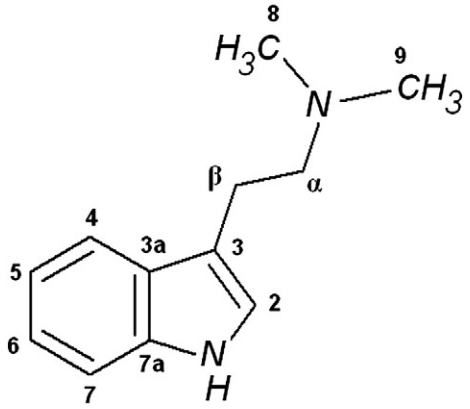

Fig. 1. Molecular structure of *N,N*-dimethyltryptamine (DMT).

and in human blood and urine [20–25]. DMT standards are often produced by synthesis of the compound from tryptamine. However, a difficulty of this procedure is the presence of impurities in the form of other tryptamine and beta-carboline derivatives [26]. Furthermore, the costs involved are high compared to those incurred when DMT is extracted from plant matrices. At our best knowledge, no combination of a fast method of extraction of DMT from the inner barks of *M. tenuiflora* and its structural characterization has been currently proposed in the literature.

The aim of this work was to optimize a method for the isolation of *N,N*-dimethyltryptamine from tissues of *M. tenuiflora*, and to apply analytical techniques for the structural characterization of the alkaloid. A purity assessment test was conducted, based on UV absorption spectrometry, with a view to using the chemical as a chromatographic analytical standard.

2. Experimental

2.1. Chemicals and reagents

GC grade *n*-hexane was purchased from Tedia (Fairfield, OH, USA). Analytical grade sodium hydroxide, sodium carbonate, and anhydrous sodium sulfate were supplied by Mallinckrodt Baker (Paris, KY, USA). Hydrochloric acid (HCl, 37%) was obtained from Vetec (Duque de Caxias, RJ, Brazil). A certified standard of tryptamine (98% purity) was purchased from Sigma-Aldrich (Somerset, NJ, USA). Deuterated chloroform ($CDCl_3$) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The chemicals were used as received, without further purification.

2.2. Sampling and preparation of plant material

Inner barks of stems and roots of *M. tenuiflora* were collected in a forest reserve located on the São Cristóvão campus of the Federal Institute of Sergipe, in northeast Brazil, between April and May 2010. A voucher specimen (ASE18817) of the material was deposited in the herbarium of the Federal University of Sergipe. The bark samples were dried at 40 °C to constant mass and powdered using a cutting mill (Model MA 048, Marconi, Piracicaba, SP, Brazil).

2.3. Isolation of N,N-dimethyltryptamine from M. tenuiflora

The powdered plant material (60 g) was suspended in 300 mL of 0.1 mol L^{-1} hydrochloric acid in a glass beaker, and sonicated in an ultrasonic bath for 24 h at a constant temperature of 25 °C. The extract was separated by simple filtration and the residual material was washed twice with 300 mL using the same acid solution. The filtrates were combined, and the solution was washed with hexane to eliminate any plant oils that might be present. The aqueous solution was basified to pH 10–11 with 0.1 mol L^{-1} NaOH, and then extracted with hexane (5 × 50 mL). The combined extracts were concentrated to dryness under reduced pressure to obtain the crude alkaloid. The solid resulting from filtration was air-dried, and recrystallized from hexane.

2.4. Structural characterization of N,N-dimethyltryptamine

2.4.1. Nuclear magnetic resonance

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 200 MHz, using $CDCl_3$ solutions. Chemical shifts were referenced to the residual solvent peak, or to tetramethylsilane (TMS) as an external reference. The data were reported in terms of the chemical shift (δ, in ppm), multiplicity, coupling constant (*J*, in Hz), and integrated intensity. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 50 MHz (using $CDCl_3$ solutions). The chemical shifts were referred to the $CDCl_3$ solvent peak. The multiplicity of a particular signal was indicated as s (singlet), d (doublet), t (triplet), or m (multiplet). The ^{1}H NMR and ^{13}C NMR spectra were measured using a Bruker Spectrospin Avance DPX-200 spectrometer (Fällanden, Switzerland).

2.4.2. Gas chromatography–mass spectrometry

GC–MS/MS analyses were performed with a Varian 3800 gas chromatograph (Varian Instruments, Sunnyvale, CA, USA) coupled to a Varian 320-MS QqQ mass spectrometer. Samples were injected into a split/splitless injector (Varian model 1177) using an autosampler (Varian model 1084). A Varian Factor Four VF-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The mass spectrometer was operated in electron ionization (EI) mode, at 70 eV. The

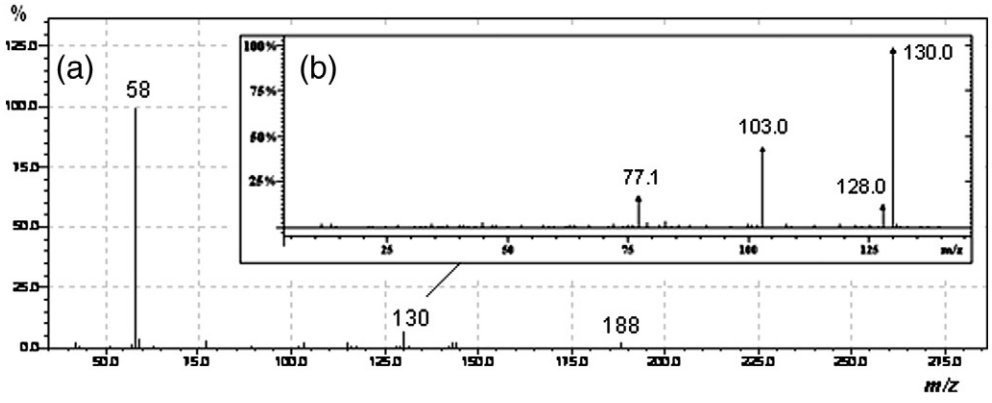

Fig. 2. GC-MS/MS (SRM mode) mass spectrum of DMT isolated from *M. tenuiflora* (a) and mass fragmentation for ion *m/z* 130 (b).

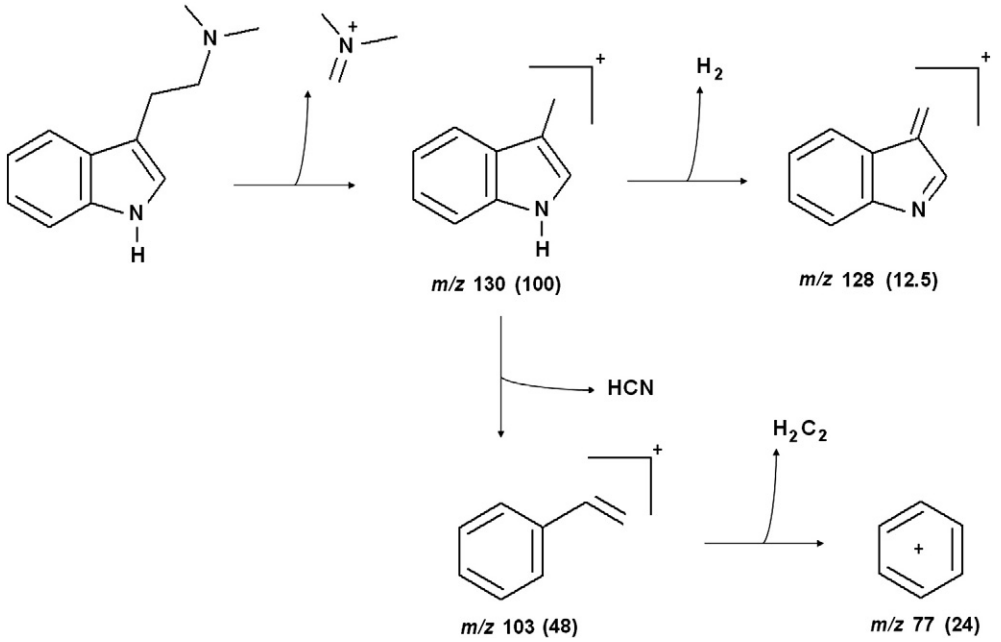

Fig. 3. Suggested fragmentation mechanism for ion *m/z* 130.

computer that controlled the system also contained an EI-MS library. The mass spectrometer was calibrated with perfluorotributylamine (PFTBA). After the ionization process, ions were passed through a hexapole ion guide to the mass analyzers (mass range is from 40 to 500 *m/z*). Helium (99.9999% purity) at a flow rate of 1.0 mL min^{-1} was used as the carrier gas and argon (99.999% purity) was employed as the collision gas at a pressure of 1.5 mTorr. Data acquisition and processing were performed with the Varian workstation software. The injector temperature was 250 °C. The oven temperature program has an initial temperature of 60 °C for 3 min, followed by a ramp to 200 °C at 8 °C min^{-1}, a further ramp to 280 °C at 10 °C min^{-1}, and a hold at 280 °C for 10 min. The QqQ mass spectrometer was operated in selected reaction monitoring (SRM) mode, and the temperatures of the transfer line, manifold, and ionization source were set at 300, 40, and 250 °C, respectively. The analysis was performed with a filament/multiplier delay of 4.0 min, in order to prevent instrument damage. The electron multiplier voltage was set at 1000 V (the value obtained in the auto-tuning process), and the scan time was 0.6 s. The total run time was 32.5 min.

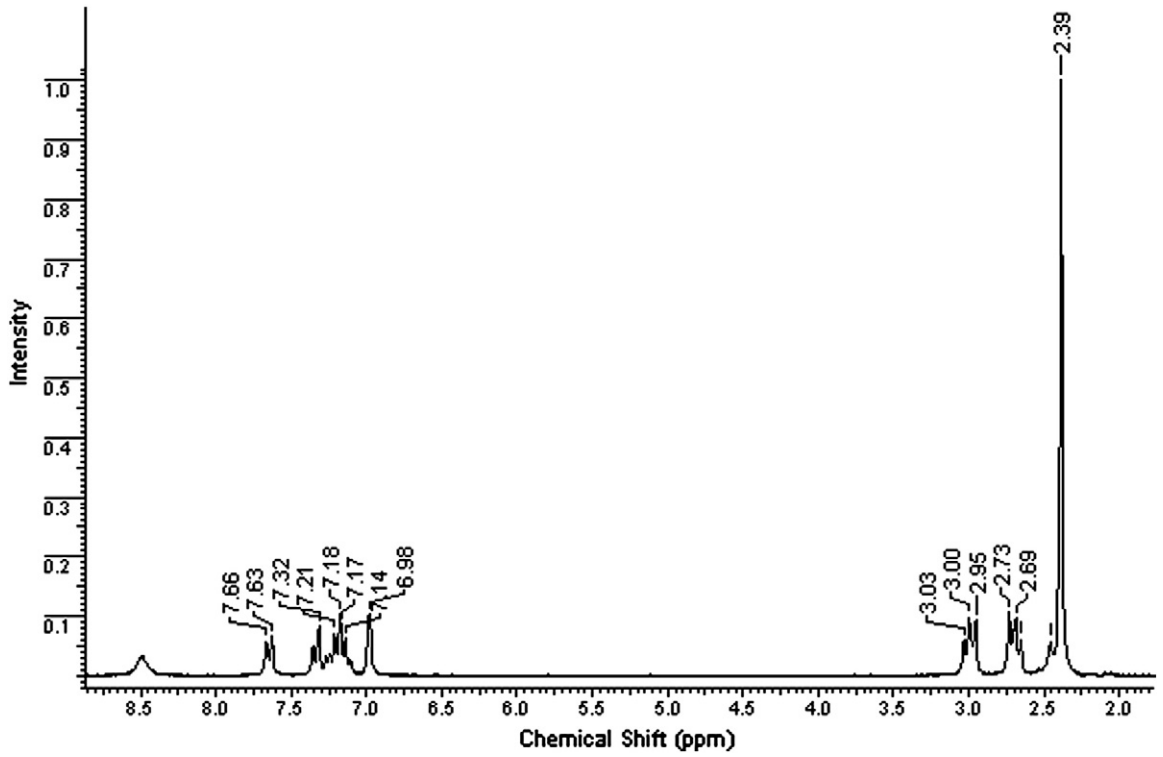

Fig. 4. ^{1}H NMR spectrum of DMT isolated from *M. tenuiflora*.

Table 1
^{13}C-NMR chemical shifts at 50 MHz of the DMT isolated from *M. tenuiflora*.

Carbon	Chemical shifts	
	Reference	Observed
C_2	122.8[a]	122.01
C_3	112.9[a]	114.37
C_{3a}	127.6[a]	127.67
C_4	118.5[a]	118.94
C_5	118.6[a]	119.28
C_6	121.1[a]	121.79
C_7	111.7[a]	111.36
C_{7a}	136.5[a]	136.55
C_β	23.7[b]	23.86
C_α	60.4[b]	60.53
C_8; C_9	45.5[b]	45.61

[a] Ref. [29].
[b] Ref. [17].

2.4.3. Mass spectrometer direct sample inlet

A Shimadzu DI-2010 direct sample inlet accessory was attached to a QP2010 GC/MS (Shimadzu, Kyoto, Japan), in order to directly introduce the sample into the mass spectrometer. The temperature was set to 340 °C.

2.4.4. Infrared and melting point measurements

Infrared spectra were recorded with a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA), in the range of 4000–400 cm^{-1}, using conventional KBr pallets. Melting points were measured in open capillary tubes, using a Mel-Temp II melting point apparatus (Laboratory Devices Inc., Menlo Park, CA, USA).

2.4.5. Ultraviolet/visible molecular absorption spectrometry

A Cary 50 UV–visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used for measurements (in triplicate, at 290 nm) of ten tryptamine standard solutions at concentrations in the range of 0.2 to 100 μg mL^{-1}, in order to generate an analytical curve. The percentage of DMT was determined using solution concentrations of 6.25, 12.5, 25.0, and 50.0 μg mL^{-1}. The measurements were performed at a wavelength of 275 nm.

3. Results and discussion

3.1. Extraction and isolation of N,N-dimethyltryptamine from M. tenuiflora

The traditional liquid–liquid procedure for extraction of indole alkaloids from plant matrices was employed [27]. The alkaloids form salts in acidic aqueous media, and show both greater solubility and enhanced stability at low pH values. In addition, the protons in acidic aqueous media assist in breaking down the sample matrix, so that the analyte is released more easily.

The acid extract was basified with sodium hydroxide, and then extracted with hexane, to give 421.4 mg of crude alkaloids (0.7% yield). Final purification was accomplished by recrystallization from hexane. The white crystals of *N,N*-dimethyltryptamine appeared after 24 h at −5 °C, and weighed 181.0 mg (0.3% yield).

3.2. Characterization of N,N-dimethyltryptamine

3.2.1. Gas chromatography–mass spectrometry analysis

An aliquot of the isolated compound was analyzed by GC/MS in full scan mode, and showed a prominent peak at 21.2 min. To confirm the identity of the compound, the spectrum of the peak (Fig. 2a) was compared with the spectra available in the Wiley electron impact mass spectrum library (Palisade Corporation, New York, USA). There was 98% similarity between the measured and library spectra, and the ions *m/z* 130 and *m/z* 58 were selected for tandem mass spectrometry. No satisfactory signal was achieved for in-source fragmentation of the *m/z* 58 ion. The mass spectrum obtained for the ion *m/z* 130 can be seen in Fig. 2b and its suggested fragmentation mechanism [28] is shown in Fig. 3.

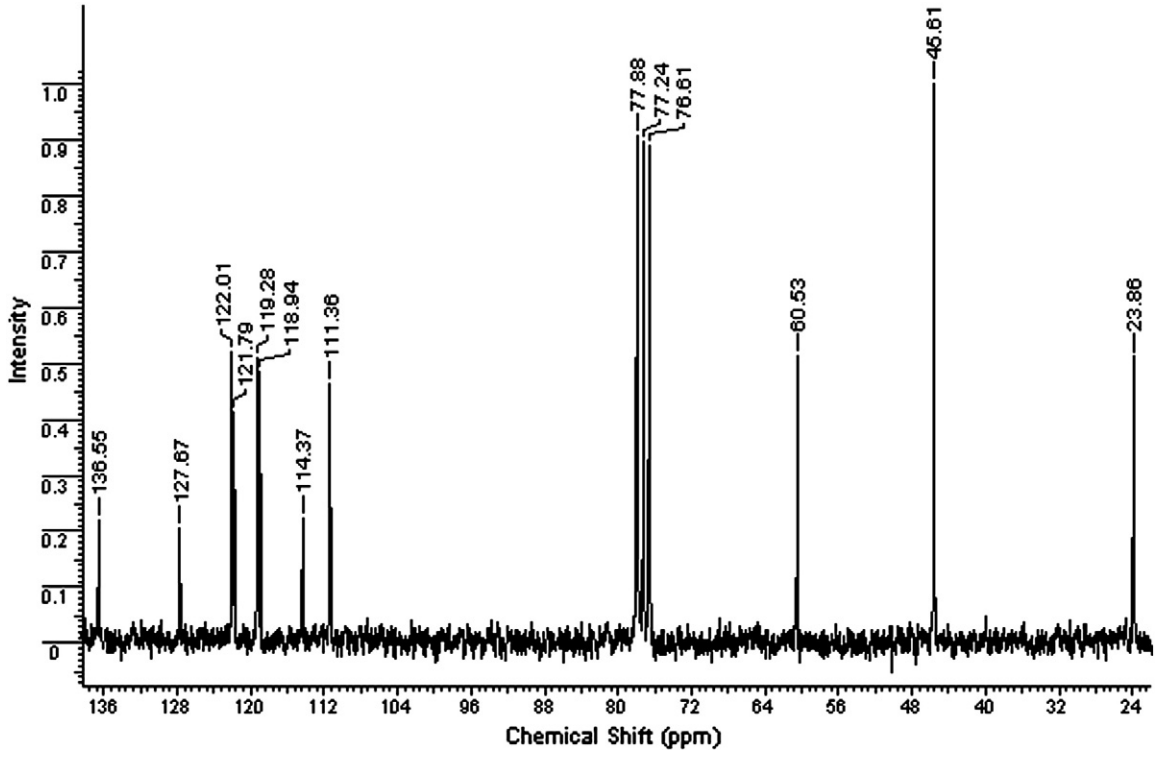

Fig. 5. ^{13}C NMR spectrum of DMT isolated from *M. tenuiflora*.

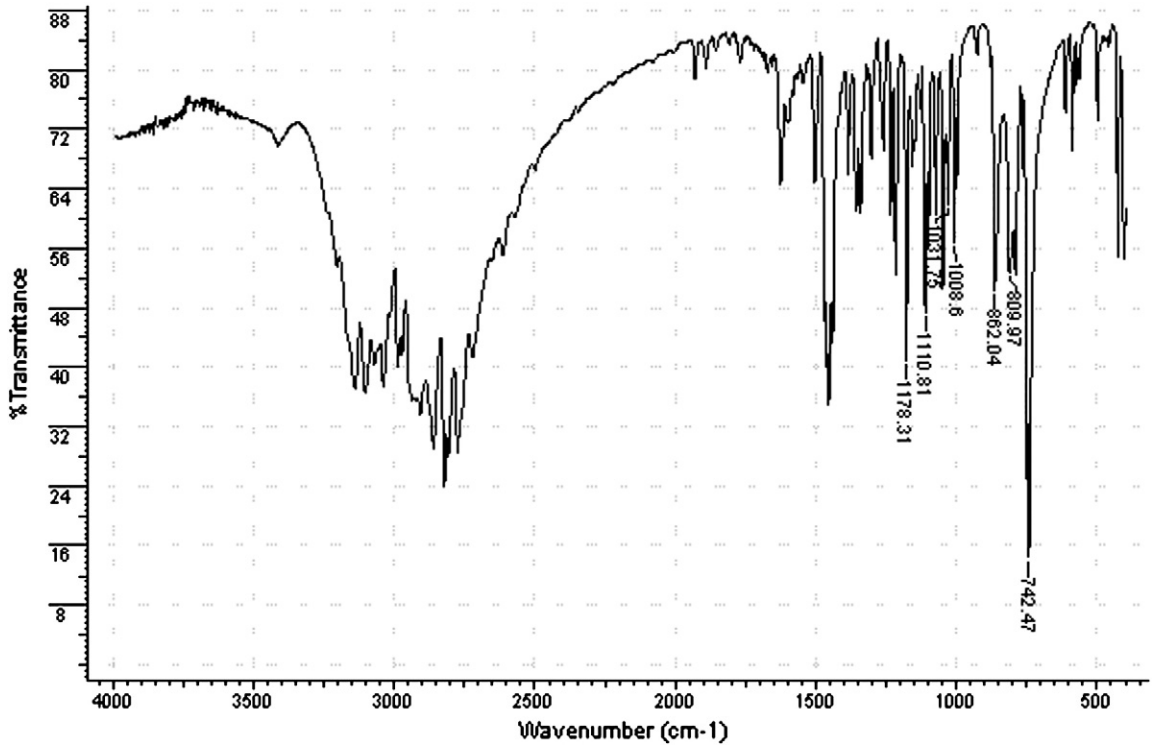

Fig. 6. Infrared spectrum of DMT isolated from *M. tenuiflora*.

3.2.2. Mass spectrometer with direct sample inlet

The direct insertion of crystals into the mass spectrometer resulted in a spectrum with a molecular ion peak at *m/z* 188, and a base peak at *m/z* 58. These and other peaks in the *N*,*N*-dimethyltryptamine spectrum were similar to the spectrum provided in the NIST Mass Spectral Database.

3.2.3. Nuclear magnetic resonance spectroscopy

The chemical shift values of the ^{1}H NMR spectrum (200 MHz, $CDCl_3$, ppm) (Fig. 4) were: 2.39 [s, 6H, N(C***H***$_3$)2]; 2.69 [br, t, 2H, $J \approx 7.0$; C***H***$_2$CH$_2$N(CH$_3$)$_2$]; 3.00 [br, t, 2H, $J \approx 7.00$, C***H***$_2$N(CH$_3$)$_2$]; 6.98 [br, s, 1H, C═***H***]; 7.10–7.39 [3H, m, Ph]; 7.65 [1H, d, $J = 7.51$, H4Ph]; and 8.49 [br, s, 1H, N***H***]. These values were in agreement with literature data [30,31]. Table 1 compares the ^{13}C NMR data obtained for DMT isolated from *M. tenuiflora* (Fig. 5) with values reported previously [17,29–31].

3.2.4. Infrared analysis

The infrared spectrum obtained for the crystals showed peaks at 742, 809, 862, 1008, 1031, 1110, and 1178 cm^{-1} (Fig. 6), which is in good agreement with the literature [32,33].

3.2.5. Melting point

The melting point of DMT recrystallized from hexane was 55.5 °C, which was compatible with the literature value (53.5–57.5 °C) [34]. The literature describes a large difference between the melting points of the DMT ranging between 44 and 68 °C. In addition, no study demonstrated an evidence of DMT crystal polymorphism [32].

3.3. Ultraviolet/visible molecular absorption spectrometry

In order to quantify DMT isolated from *M. tenuiflora*, it was assumed that tryptamine shows the same molar absorbance at 290 nm as *N*,*N*-dimethyltryptamine at 275 nm, when these compounds are present in methanol [35]. The data revealed a DMT content of 100.19 ± 4.91% (i.e. higher than 95%). This was corroborated by a DMT analytical curve constructed at 275 nm, using the absorbance of eight DMT standard solutions at concentrations between 1.56×10^{-3} and 0.1 mg mL^{-1}. Good agreement was obtained between the equations obtained for tryptamine at 290 nm ($y = 28.798x + 0.031$, $r = 0.9968$) and for *N*,*N*-dimethyltryptamine at 275 nm ($y = 29.844x + 0.015$, $r = 0.9998$).

4. Conclusion

A simple and rapid acid–base extraction method was employed for the isolation of *N*,*N*-dimethyltryptamine from *M. tenuiflora* stems and roots, resulting in the formation of white crystals with a purity level higher than 95%, which were structurally characterized using ^{1}H NMR and ^{13}C NMR, MS, FTIR, and UV/vis absorption techniques. The high purity of the *N*,*N*-dimethyltryptamine obtained by this method enables it to be used as an analytical standard.

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