The Isolation, Identification, and Quantitation of Dimethyltryptamine (DMT) in *Mimosa hostilis*

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ABSTRACT: Dimethyltryptamine (DMT) was extracted from the root bark of *Mimosa hostilis* via three methods, using methanol (direct or via Soxhlet) and acetic acid (direct only), respectively. The product from the direct methanol extraction was used in both qualitative and quantitative analysis, while the product from the acetic acid extraction (isolated in crystal form after workup) was used for qualitative analysis. FTIR/ATR, GC/MS, GC/IRD, ^{1}H-NMR, and HPLC data are presented. Quantitative analysis by ^{1}H-NMR and HPLC indicated 0.9 percent and 0.8 percent DMT, respectively, in the analyzed samples.

KEYWORDS: *Mimosa hostilis*, Dimethyltryptamine, DMT, Extraction, Analysis, Forensic Chemistry

Introduction

Tryptamines are substituted indole compounds which are both naturally occurring and synthetically manufactured. Many tryptamines, including dimethyltryptamine (DMT, Figure 1), have hallucinogen properties, and are therefore listed as Schedule I drugs under the U.S. Controlled Substances Act (21 CFR 1308.11). DMT is present in many plants and their seeds, including in *Mimosa hostilis* and *Psychotria viridis* [1-3], and can be abused by smoking, injection, or ingestion of either these natural materials or their crude or purified extracts, either alone or in combination with other extracts (e.g., Ayahuasca [4].) *Mimosa hostilis* and similar natural plant materials are not formally controlled (by name) in the United States; however, they are controlled (Schedule I) if they are shown to contain DMT or other controlled hallucinogens. Despite their controlled status, a number of DMT-containing natural products, including *Mimosa hostilis*, are openly marketed on the Internet.

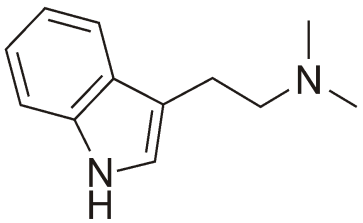

Figure 1. Structure of Dimethyltryptamine (DMT; $C_{12}H_{16}N_2$, m.w. = 188.27).

Clandestine DMT extraction laboratories are occasionally seized by law enforcement agencies [e.g., 5]. The basis of this report was the seizure of an unknown plant material (Photo 1) at a clandestine MDMA (Ecstasy) laboratory in rural Pennsylvania. GC/MS analysis of a methanolic extraction of the material identified DMT. Upon debriefing, the defendant in the case indicated that material was root bark from *Mimosa hostilis*. Similar seizures of this material have been made at other clandestine laboratory sites in the United States, and subsequent analyses of those exhibits confirmed that they also contained DMT.

Photo 1. *Mimosa hostilis* Root Bark Seized at Clandestine Lab in Pennsylvania.

Experimental

Methanol Extraction: The root bark was cut into small pieces then ground in a blender to produce a very fine powder. For direct extraction, methanol was added to the powder, heated to 60^{O}C with stirring for 1 hour, and then filtered. This step was repeated three more times, except the re-extractions were carried out for only 5 - 10 minutes each. The combined extracts were evaporated to a residue over steam, then reconstituted as needed for analysis. For Soxhlet extraction, the powdered material was placed in an extraction thimble, placed in a Soxhlet, and extracted with 50 mL of methanol for approximately 50 volumes. The solvent was evaporated to a residue over steam, then reconstituted as needed for analysis.

Acetic Acid Extraction: The root bark was cut into small pieces then ground in a blender to produce a very fine powder. A 3% acetic acid solution was added to the powder, and the resulting suspension was stirred for approximately two hours. The solution was filtered and transferred to a separatory funnel, made basic with sodium hydroxide, and then extracted with methylene chloride. The methylene chloride solution was isolated, and the aqueous later was re-extracted with a second volume of methylene chloride. The combined extracts were dried over magnesium sulfate, filtered, and evaporated to give a crystalline material.

Fourier Transform Infrared with Attenuated Total Reflectance (FTIR/ATR)
Instrument: Perkin-Elmer Spectrum One FTIR.
Data collection: Four scans were collected between 650 cm^{-1} and 4000 cm^{-1}.
Resolution: 4 cm^{-1}.
Sample: Crystals from the acetic acid extraction.

Gas Chromatograph/Mass Spectrometer (GC/MS)
Instrument: Agilent 6890N GC/Agilent 5973 Mass Selective Detector.
Column: HP-5, 30 m x 0.25 mm x 0.25 μm column.
Temperature program: 90^{O}C - 120^{O}C @ 35^{O}C/min; initial time 1.35 min, then 120^{O}C - 290^{O}C @45^{O}C/min; initial time 0.55 min, final hold time 8.5 min.
Injection port temperature: 300^{O}C.
Transfer line temperature: 280^{O}C.
Ionization source: Electron ionization (EI).
Mass analyzer: Quadrupole.
Scan range: 40 - 525.
Quadrupole temperature: 150^{O}C.
MS source temperature: 230^{O}C.
Sample preparation: Residue from the methanol extraction, reconstituted in methanol.

Gas Chromatograph/Infrared Detector (GC/IRD)
Instrument: Agilent 6890 GC/Varian IRD Detector.
Column: HP-5, 25 m x 320 μm x 0.52 μm column.
Split mode: 5:1.
Temperature program: 100^{O}C for 1.50 min, ramp @ 35^{O}C/min to 120^{O}C, hold for 0.55 min, then ramp @ 40^{O}C/min to 290^{O}C, final hold for 8.13 min.
Inlet temperature: 270^{O}C.
Injection volume: 2 μL.
Constant column flow: 2.0 mL/min.
Transfer line temperature: 280^{O}C.
Flow cell temperature: 280^{O}C.
KBr windows.
Optical resolution: 8.
1.5 scans/sec.
Sample: Residue from the methanol extraction, reconstituted in chloroform.

Proton Nuclear Magnetic Resonance (^{1}H-NMR)
Instrument: Mercury 400 MHz.
Number of transients: 8.
Relaxation delay: 45 seconds.
Pulse: 90^{O}.
Sweep width: 6393.9 Hz.
Temperature: 25^{O}C.
Sample preparation for qualitative analysis: Crystals from the acetic acid extraction, reconstituted in 1 mL CD_3OD.
Sample preparation for quantitative analysis: 5.0 g *Mimosa hostilis* extracted via the methanol extraction procedure, yielding 1.52 g residue. Added 28.0 mg to 1 mL CD_3OD, with 5.544 mg maleic acid added as the internal standard.

High Performance Liquid Chromatography (HPLC)
Instrument: Agilent 1100 Series HPLC.
Column: Phenomonex Partisil 5μm ODS-3 (C-18).
Mobile phase: Phosphate buffer pH 2.5:methanol (90:10).
Injection: 5 μL.
Flow rate: 1.0 mL/min.
Detection: 280 nm.
Run time: 8 minutes.
Sample preparation: 9.9 g *Mimosa hostilis* extracted via methanol extraction procedure, with the residue reconstituted in 100 mL methanol.

Results and Discussion

The extraction of DMT from *Mimosa hostilis* was completed using two different solvents, methanol (direct or via Soxhlet) and acetic acid (direct only). The methanol extraction gave the maximum recovery of DMT for qualitative and quantitative analysis; however, the extract included other soluble plant impurities. The extraction efficiency using methanol was identical whether done directly or via Soxhlet. The acetic acid extraction gave a very clean, pure product, but in lower yield versus the methanol extraction.

FTIR/ATR: The crystals from the acetic acid extraction procedure produced a clean spectrum (Figure 2).

GC/MS: DMT eluted at 6.06 minutes using the described method. The spectra showed a base peak at *m/z* = 58 and the molecular ion at *m/z* = 188, along with smaller peaks at *m/z* = 44, 77, and 130 (Figures 3 and 4).

GC/IRD: DMT eluted at 6.88 minutes using the described method (Figure 5).

^{1}H-NMR (Qualitative): The singlet at 2.35 ppm is due to the two N-methyl groups, the two triplets at 2.70 ppm and 2.95 ppm correspond to the *alpha* and *beta* methylene groups. The multiplet at 7.00 ppm corresponds to protons 2, 5, and 6 on the indole. Finally, the two doublets at 7.25 ppm and 7.50 ppm correspond to protons 4 and 7 on the indole. A slight shift was observed in the extract versus a DMT standard; this was due to pH differences (the spectrum was obtained from DMT acquired using the acetic acid extraction procedure, which involved an acid base workup). (Figure 6). (Quantitative): Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in *Mimosa hostilis* (Figure 7 and Table 1). Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in Mimosa hostilis (Figure 8 and Table 2).

HPLC: DMT eluted in under 3 minutes. Using the methanol extract, DMT was determined to be 0.8% weight/weight in *Mimosa hostilis* (Figure 9 and Table 3).

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* Law Enforcement Restricted Publication.

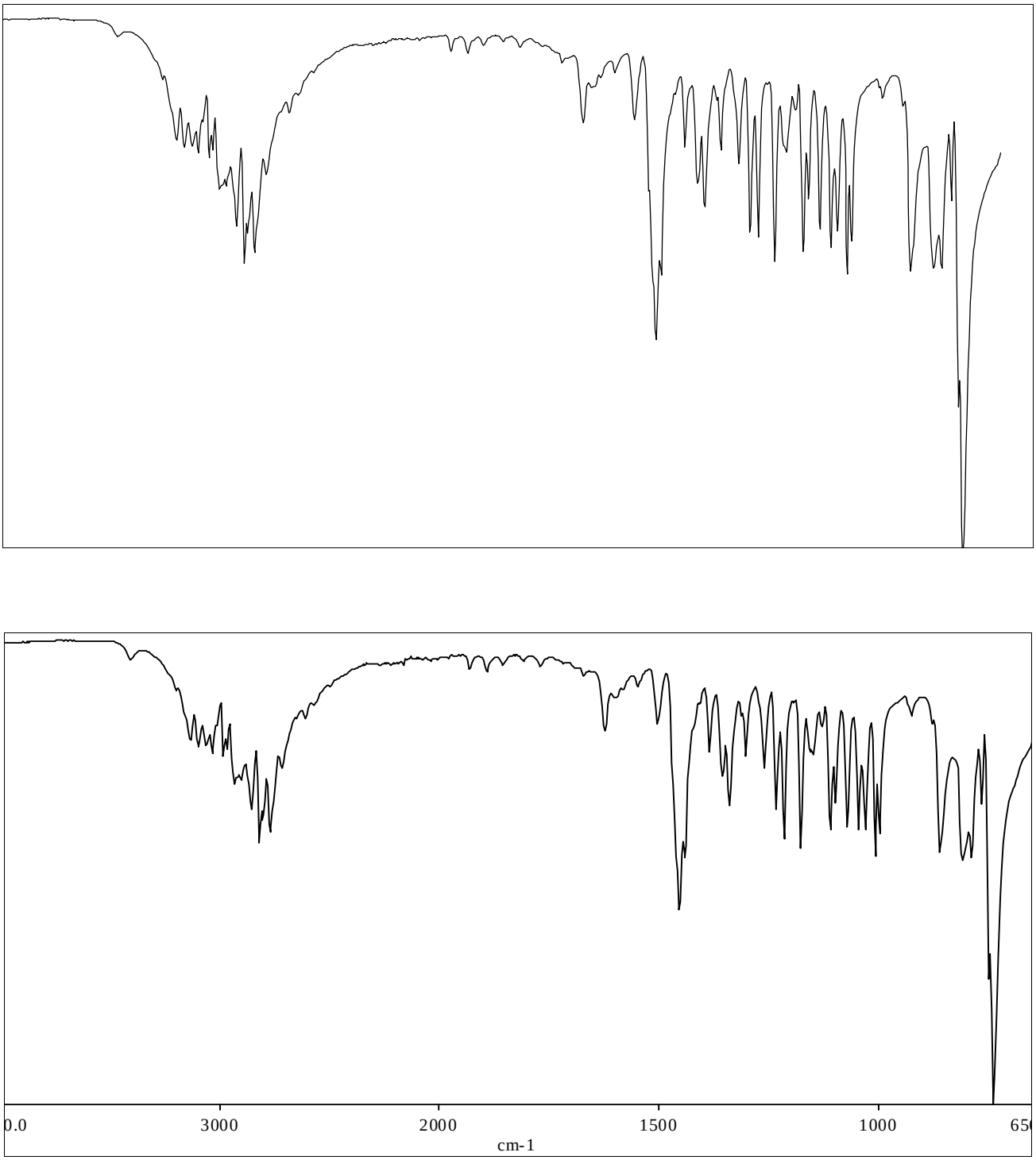

Figure 2. FTIR/ATR of a DMT Standard (Top Trace) and DMT from the Acetic Acid Extraction Procedure (Bottom Trace). [Note: The DMT Standard was Recrystallized from Chloroform.]

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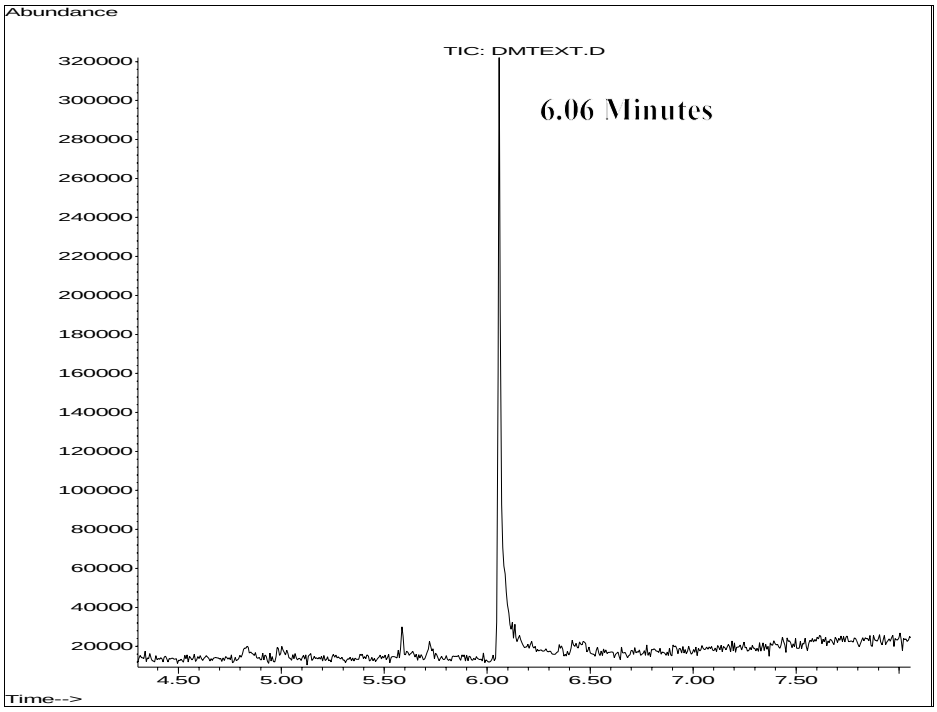


Figure 3. GC/MS Total Ion Chromatogram of DMT (Methanol Extract).

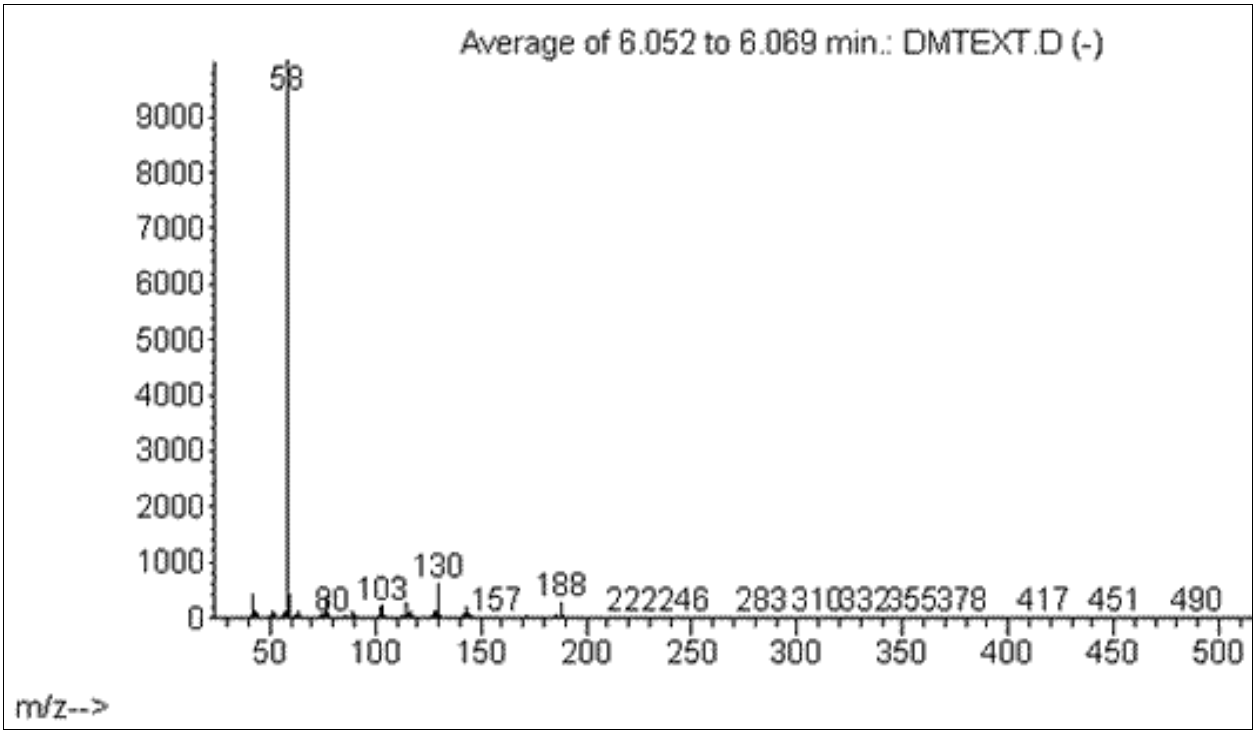
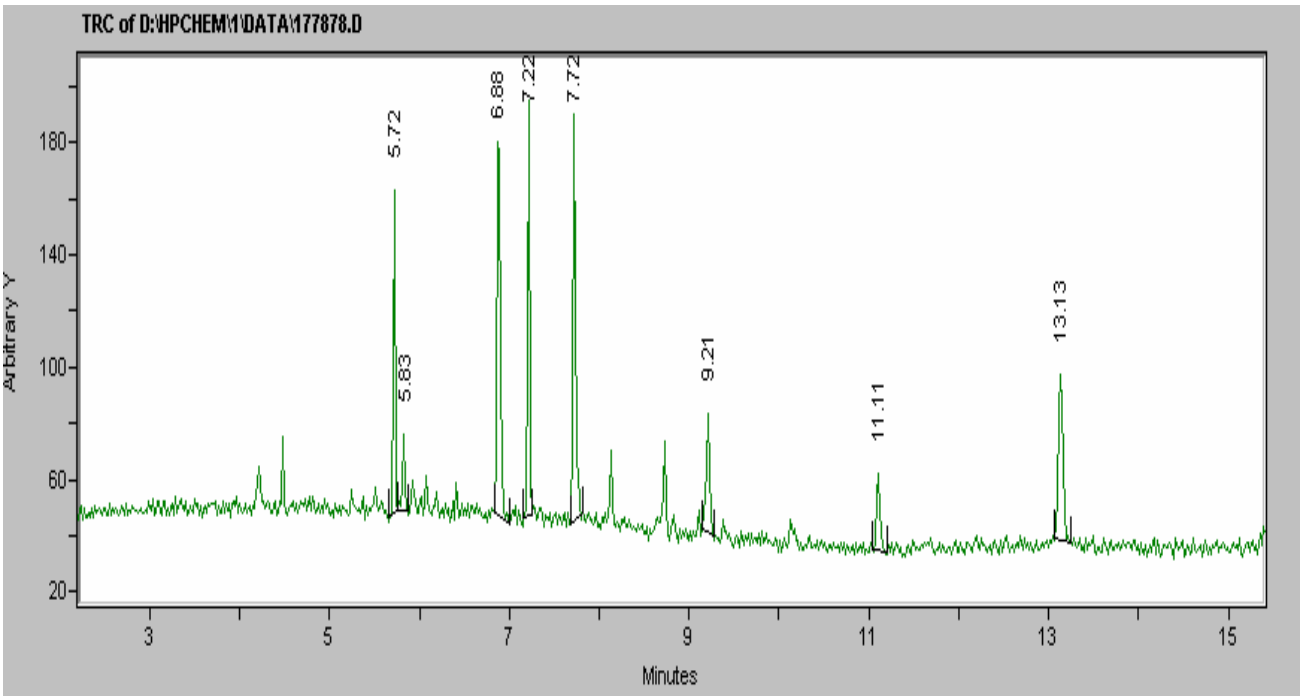


Figure 4. GC/MS Data of DMT (Methanol Extract). [Note: Molecular Ion at *m/z* = 188.]



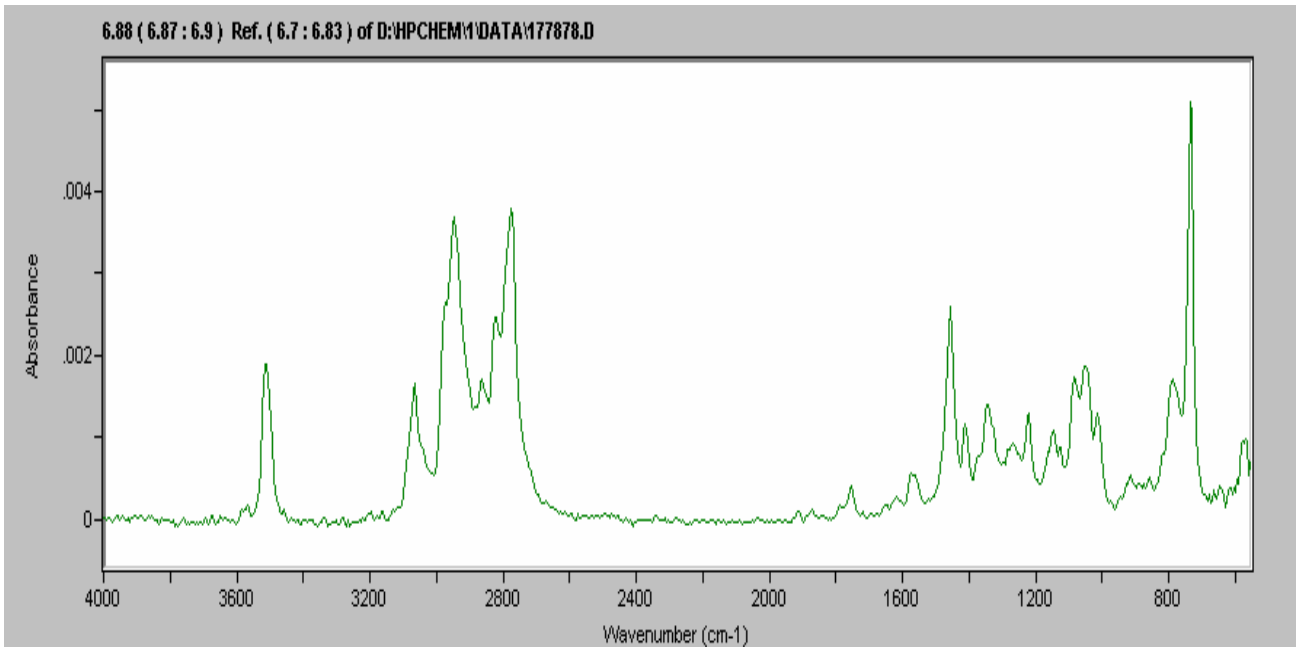


Figure 5. GC/IRD Data of DMT (Methanol Extract). [Note: DMT eluted at 6.88 Minutes.]

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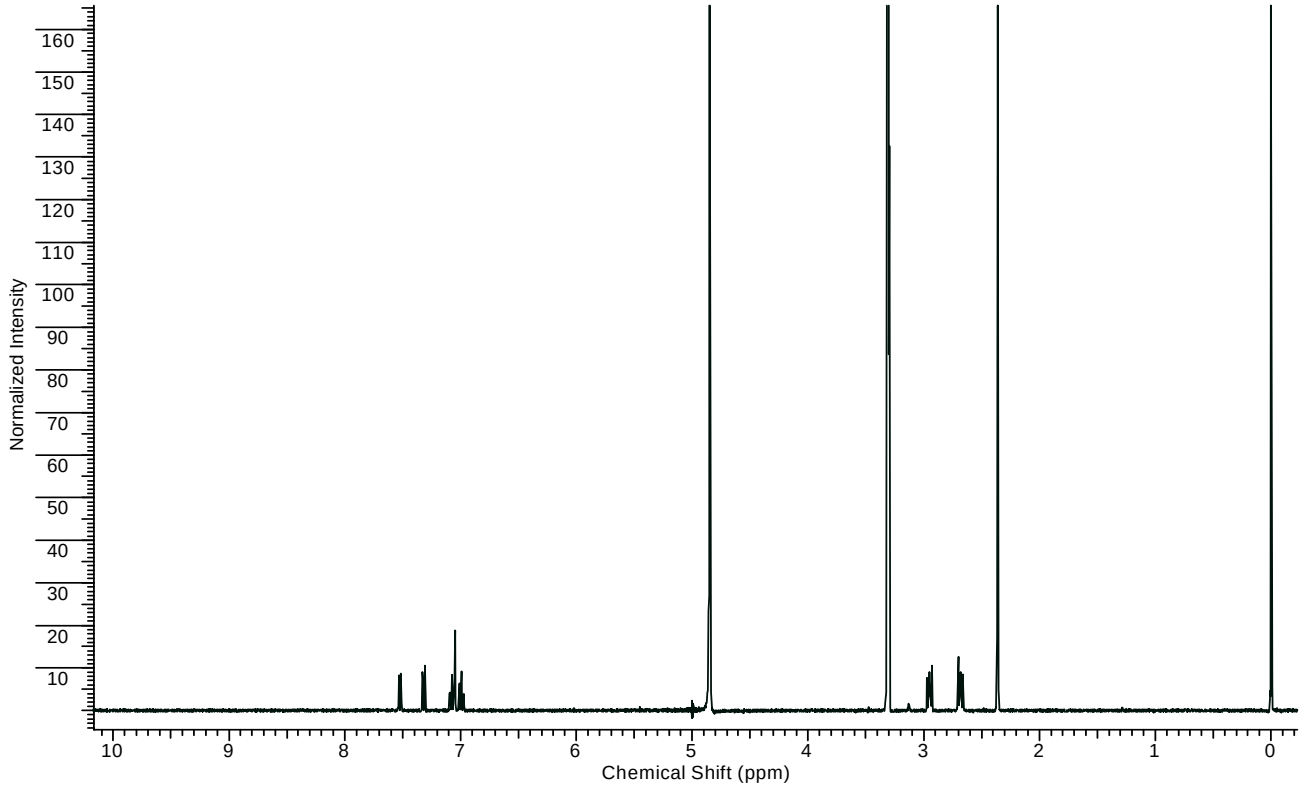

Figure 6a. Full-Scale NMR Data of DMT (Acetic Acid Extract).

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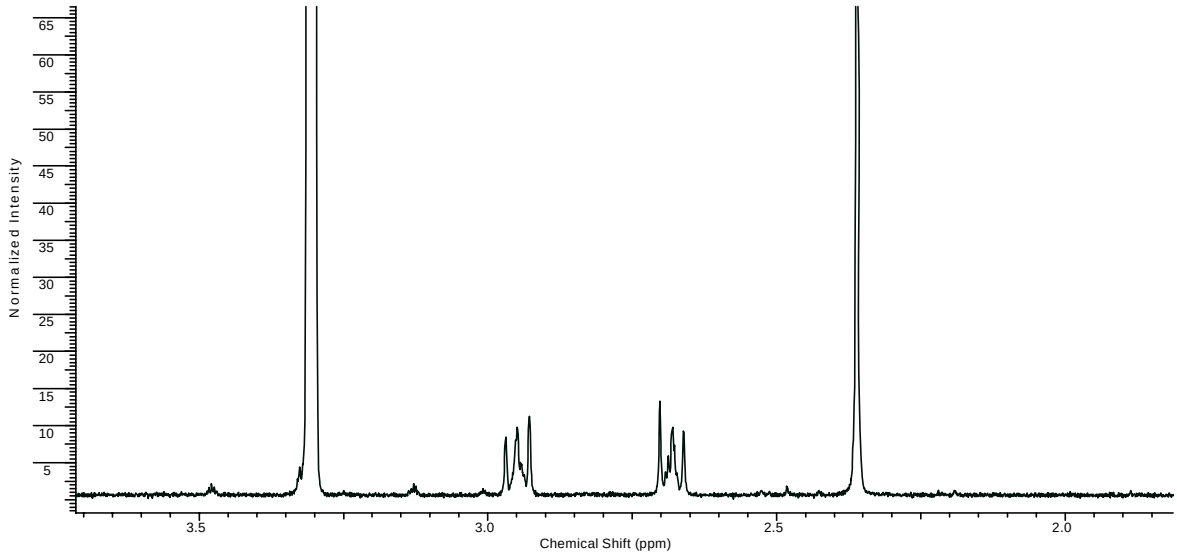

Figure 6b. Expanded Spectrum from 2 to 4 ppm. See Results and Discussion for Peak Assignments.

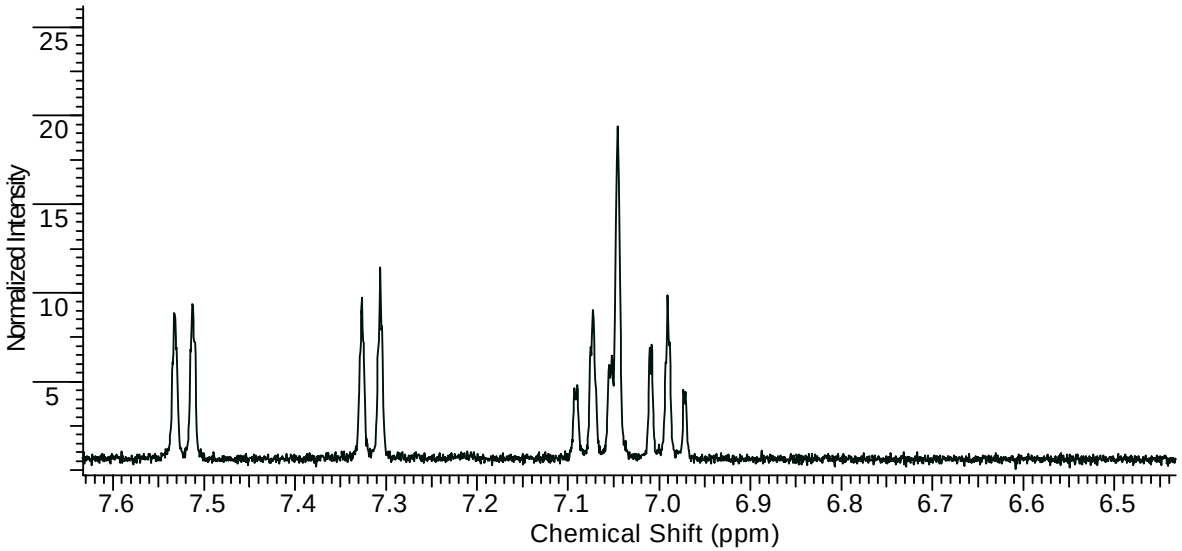

Figure 6c. Expanded Spectrum from 6.5 to 7.6 ppm. See Results and Discussion for Peak Assignments.

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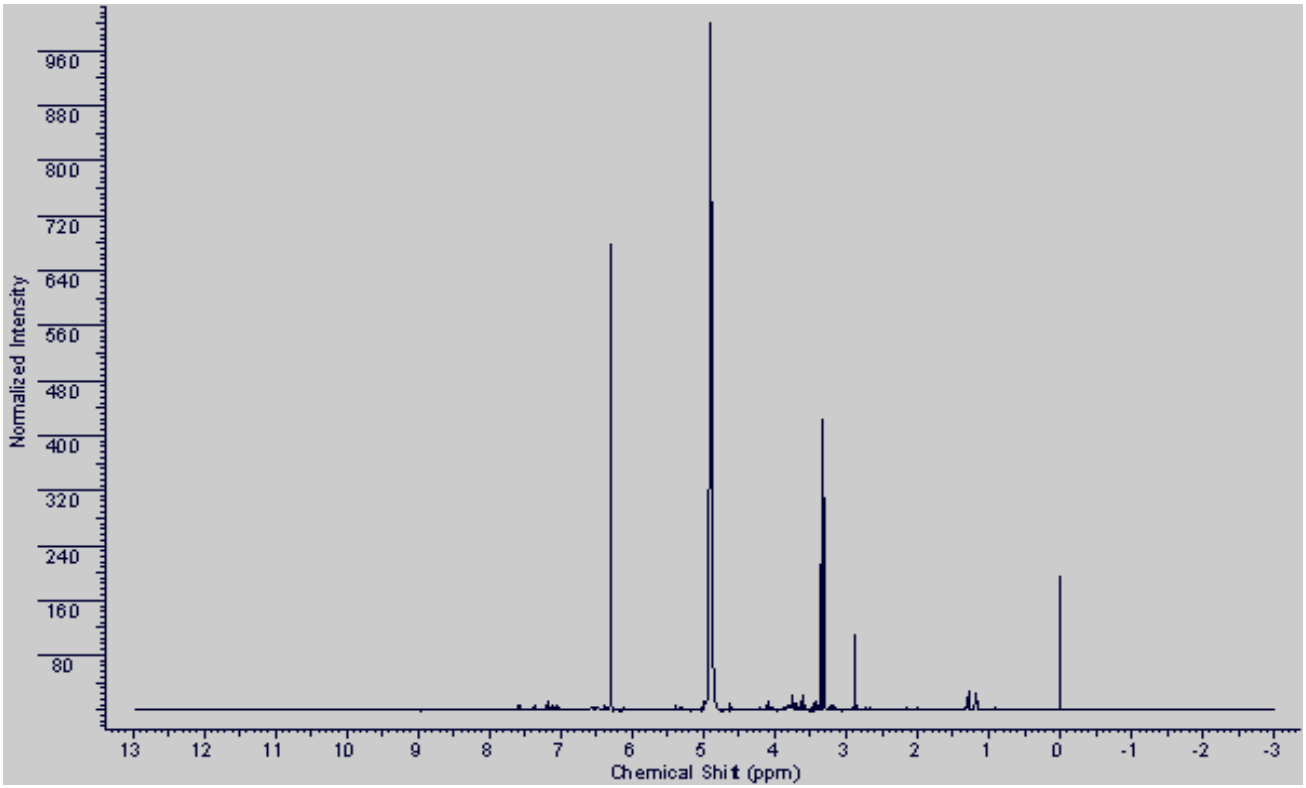

Figure 7a. NMR Quantitation of DMT (Direct Methanol Extract); See Table 1.

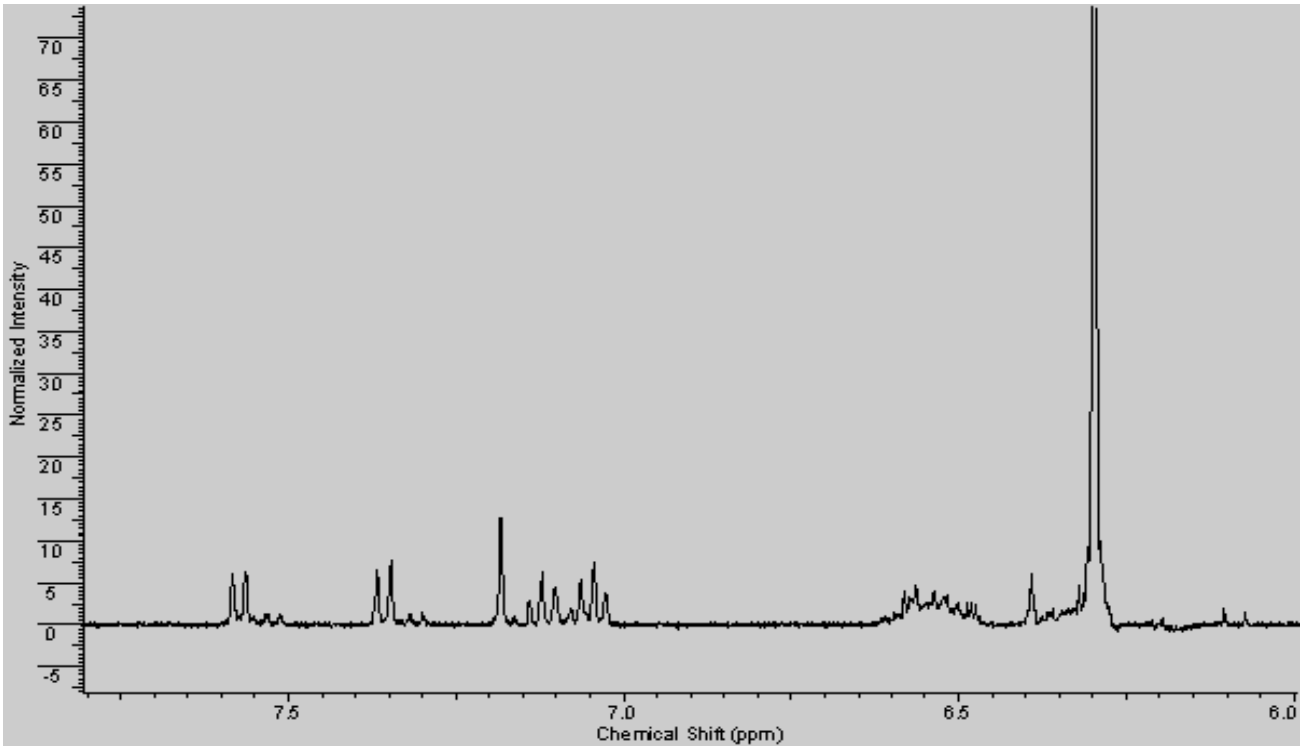

Figure 7b. NMR Quantitation of DMT (Direct Methanol Extract); Expansion; See Table 1.

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Table 1. NMR Quantitation of DMT (Direct Methanol Extract); See Figure 7.					
Original Amount of Plant Material (g)	5.0				
Amount of extraction product (g)	1.52				
Sample Amount (mg)	28.00				
Molecular Weight of Sample	188.3				
Solvent	CD3OD				
Internal Standard (I.S.)	Maleic Acid				
Molecular Weight of I.S.	116.07				
I.S. Amount (mg)	5.544				
Peaks Chemical Shift (ppm)	[6.26..6.34]	[7.02..7.08]	[7.10..7.15]	[7.34..7.42]	[7.54..7.62]
Integral Value	34.12	2.13	1.64	1.39	1.27
Number of protons represented	2	1	1	1	1
Quantitation Value	Internal Standard	4.01%	3.09%	2.61%	2.39%
Average purity of extracted material	3.03%				
Amount of DMT in *Mimosa hostilis*	0.918%				

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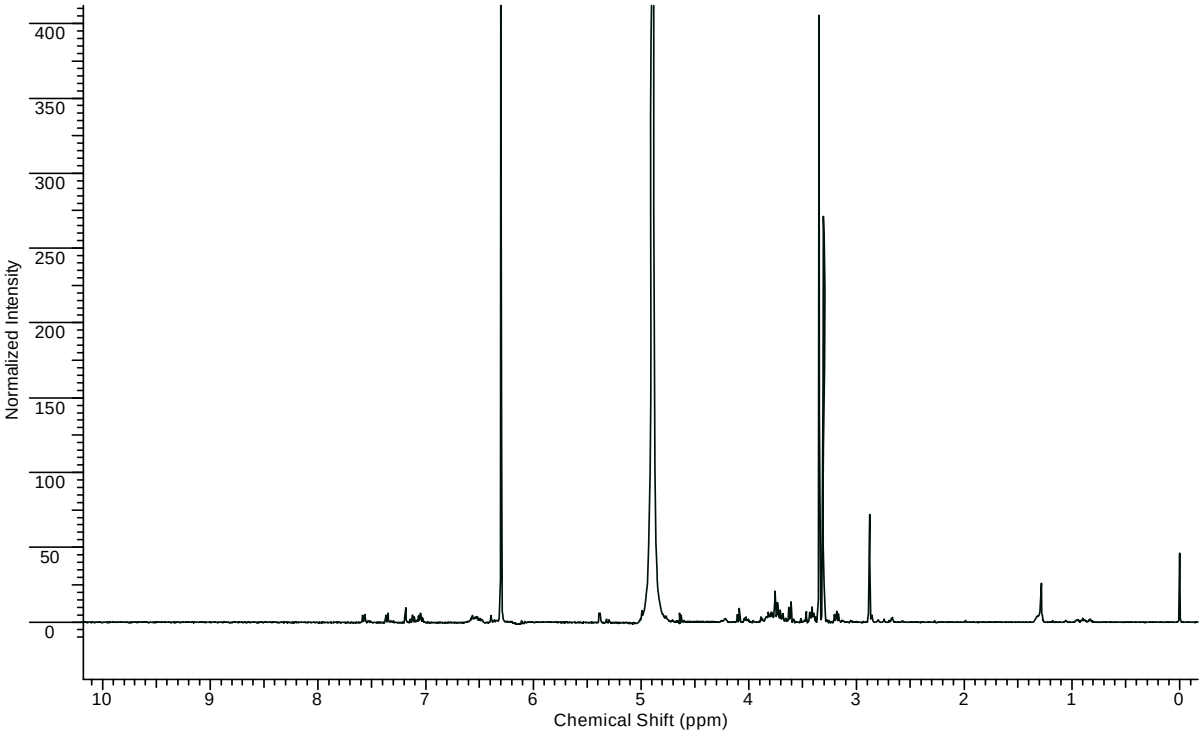

Figure 8a. NMR Quantitation of DMT (Methanol - Soxhlet Extract); See Table 2.

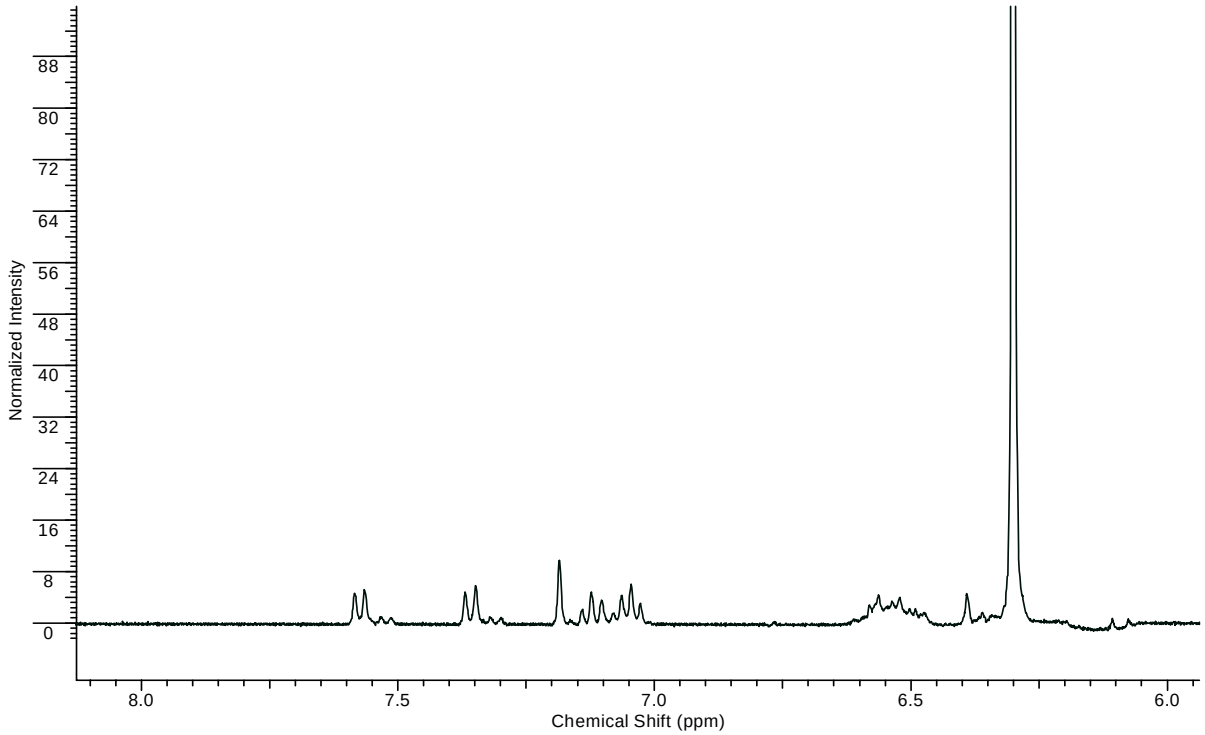

Figure 8b. NMR Quantitation of DMT (Methanol - Soxhlet Extract); Expansion; See Table 2.

Table 2. NMR Quantitation Results of DMT (Soxhlet Extract); See Figure 8				
Sample Amount (mg)	10450.0			
Molecular Weight of Sample	188.3			
Solvent	CD3OD			
Internal Standard (I.S.)	Maleic Acid			
Molecular Weight of I.S.	116.07			
I.S. Amount (mg)	6.118			
Peaks Chemical Shift (ppm)	[6.25..6.33]	[7.01..7.17]	[7.33..7.39]	[7.55..7.61]
Integral Value	32.58	2.575	1.032	0.999
Number of protons represented	2	1	1	1
Quantitation Value	Internal Standard	1.501%	0.601%	0.582%
Amount DMT in *Mimosa hostilis*	0.894%			

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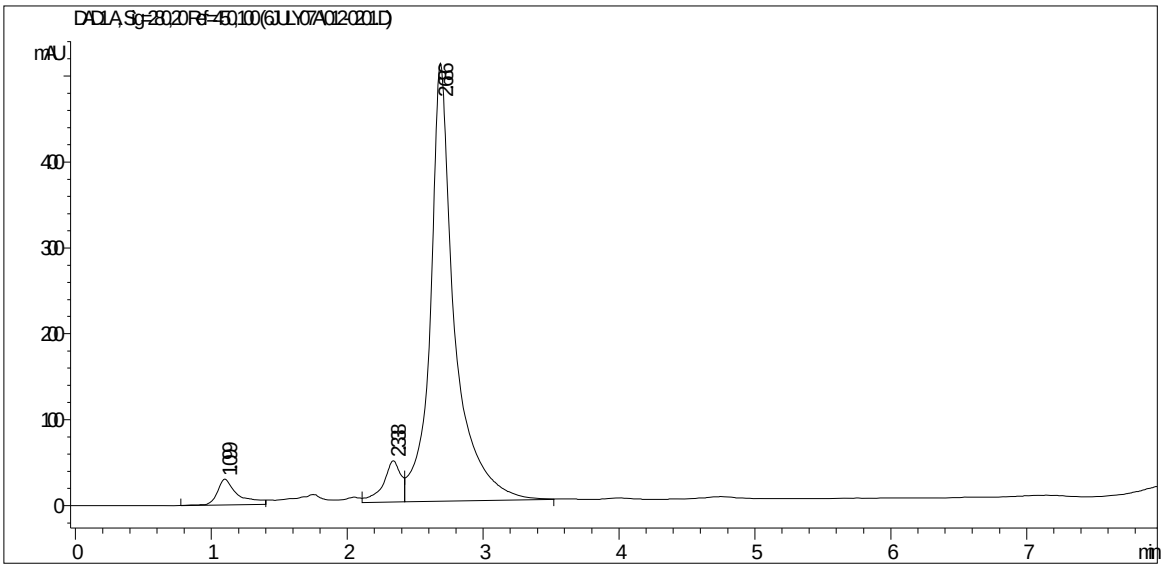

Figure 9. HPLC Quantitation of DMT (Methanol Extract); See Table 2.

Table 3. HPLC Quantitation of DMT (Methanol Extract); See Figure 9.							
	Concentration (mg/ml)	RT 1 (minutes)	RT 2 (minutes)	Area 1	Area 2	Average Area	Quant Value
DMT Standard	0.2924	2.685	2.702	2304.823	2366.984	2335.904	100.00
DMT Extract	99.00	2.686	2.688	6302.684	6339.690	6321.187	0.799