2021 Eastern Analytical CHEMISTRY, & PERSEVERANCE Symposium & Exposition CELEBRATING **60 YEARS OF EAS**



CURIOSITY,

Crowne Plaza Princeton Conference Center Plainsboro, NJ November 15-17, 2021

HPTLC/HPLC/MS ANALYSIS OF AYAHUASCA PREDICTIVE ANALYTICS

> AUTHOR/PRESENTER: SIDNEY SUDBERG **ALKEMIST LABS** SIDNEY@ALKEMIST.COM MONDAY, NOVEMBER 15TH, 2021 9:30 AM EST

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11/15/2021

What is Ayahuasca?

- Ayahuasca is a beverage prepared by boiling—or soaking—the bark and stems of Banisteriopsis caapi together with various admixture plants. (Schultes, 1957)⁽¹⁾
- The admixture employed most commonly is the Rubiaceous genus *Psychotria*, particularly *Psychotria viridis*.
- The leaves of *P. viridis* contain indole alkaloids, which are necessary for the psychoactive effect.
- Ayahuasca is unique in that its pharmacological activity is dependent on a synergistic interaction between the active alkaloids in the plants. (McKenna, 2004)⁽²⁾
- One of the components, the bark of *B. caapi*, contains B-carboline alkaloids, which are potent monoamine oxidase-A (MAOA) inhibitors;
- The other component, the leaves of *P. viridis* or related species, contains the potent short-acting hallucinogenic agent <u>N,N-dimethyltryptamine (DMT</u>).
- DMT is not orally active when ingested by itself, but can be rendered orally active when ingested in the presence of peripheral MAO inhibitors, such as the <u>B-carbolines</u>. This interaction is the basis of the psychotropic action of ayahuasca (McKenna et al., 1984).⁽³⁾

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Goals of this Study:

To discover an ideal method of prep to create a predictably higher quality medicine.

- Weight vs Volume preparation of extracts & the affect on the contents of the primary compounds of interest?
- To discover an ideal target ratio of active cpds, that can be optimized by the method &/or conditions of preparation?

Demonstrate a Fit-for-Purpose use of HPTLC analysis for the study of Natural Products, since it provides reproducible data & scientific rationale for Natural Products Research, based on its' ability to reveal the 'bigger picture', while other instrumental methods can be too sensitive & miss the 'Forest for the Trees'.

- HPTLC provides high quality data vs large quantities of data that many other methods of analysis generate.
 - Where less is more \rightarrow Lumper vs Splitter concept in Botany

ACTIVE COMPOUNDS IN AYAHUASCA:



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Tetrahydroharmine MW: 216.3 g/mol



Harmine, MW: 212.3 g/mol



Harmaline, MW: 214.3 g/mol



ACTIVE COMPOUNDS IN AYAHUASCA:



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N, N Dimethyltryptamine (DMT) MW: 188.3 g/mol P. viridis



10

12

13

14

15

0.1 Harmol+

Harmalo

Fingerprinting by HPTLC

Lane 1: Harmol, Harmalol, THH, Harmaline, Harmine, DMT (in order of increasing R_f) Lane 2: *B. caapi* KD20099BD Lane 3: *B. caapi* KD13702KT1 Lane 4: *B. caapi* 20205YCV Lane 5: *B. caapi* 20210LSN Lane 6: Harmol, Harmalol, THH, Harmaline, Harmine, DMT (in order of increasing R_f) Lane 7: P. viridis MK13001BD Lane 8: P. viridis 20205XJO Lane 9: P. ligustrifolia GV13702KT Lane 10: P. nervosa GW13702KT Lane 11: P. punctata GX13702KT Lane 12: P. alba GY13702KT Lane 13: D. cabrerana DW13702KT Lane 14: *M. hostilis* HZ13702KT Lane 15: Harmol, Harmalol, THH, 0.2 Harmaline, Harmine, DMT (in order of increasing R_f)



Fingerprinting by HPTLC

Lane 1: Harmol, Harmalol, THH, Harmaline, Harmine, DMT (in order of increasing R_f) Lane 2: *B. caapi* KD20099BD Lane 3: *B. caapi* KD13702KT1 Lane 4: B. caapi 20205YCV Lane 5: *B. caapi* 20210LSN Lane 6: Harmol, Harmalol, THH, Harmaline, Harmine, DMT (in order of increasing R_f) Lane 7: P. viridis MK13001BD Lane 8: P. viridis 20205XJO Lane 9: P. ligustrifolia GV13702KT Lane 10: P. nervosa GW13702KT Lane 11: P. punctata GX13702KT Lane 12: *P. alba* GY13702KT Lane 13: D. cabrerana DW13702KT Lane 14: *M. hostilis* HZ13702KT Lane 15: Harmol, Harmalol, THH, Harmaline, Harmine, DMT (in order of increasing R_f)

11/15/2021

Alkemist Labs: Botanical Test Method



Identification of Ayahuasca (Banisteriopsis caapi vine) and Chacruna (Psychotria viridis leaf) by HPTLC

Critical Control Point:

(Image 1) UV 254 nm

Dated: N/A Appropriate voucher reference samples, if available, or equivalent for Banisteriopsis caapi vine and Psychotria viridis leaf are essential for identification. Reference standards for harmine, harmaline, tetrahydroharmine, harmol, harmalol, and N,Ndimethyltryptamine are essential for system suitability and evaluation.

Typical Chromatograms





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Replaces Revision #: N/A

Document Control #: BTM-715-0625

7 2 2 4 5 6 7 8 8 10 11 12 13 14 15 (Image 3) Derivatized with Anisaldehyde Reagent. Visible light

Lane 1: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT,

Lane 2: Banisteriopsis caapi vine KD20099BD (1 µL) BRM

Lane 3: Banisteriopsis caapi vine KD13702KT1 (1 µL) BRM

Lane 5: Banisteriopsis caapi water extract 20210LSN (1 µL) TS

Lane 6: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT,

Lane 4: Banisteriopsis caopi vine 20205YCV (1 µL) TS

Lane 7: Psychotria viridis leaf MK13001BD (1 µL) BRM

with increasing Rr) (1µL) Reference Standards

with increasing R() (1µL) Reference Standards



(Image 4) Derivatized with Anisaldehyde Reagent. UV 366 nm

Lane 8: Psychotria viridis leaf 20205XJO (1 µL) TS Lane 9: Psychotria ligustrifolia leaf GV13702KT (1 µL) BRM Lane 10: Psychotria nervosa leaf GW13702KT (1 µL) BRM Lane 11: Psychotria punctata leaf GX13702KT (1 µL) BRM Lane 12: Psychotria alba leaf GY13702KT (1 µL) BRM Lane 13: Diploptervis cabrerana leaf DW13702KT (1 µL) BRM Lane 14: Mimosa hostilis leaf HZ13702KT (1 µL) BRM Lane 15: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT, with increasing Rf) (1µL) Reference Standards

System Suitability:

Images 1-4:

Harmol and Harmalol reference standard bands are typically present at approximately Rf = 0.04. Tetrahydroharmine (THH) reference standard band is typically present at approximately $R_f = 0.21$. Harmaline reference standard band is typically present at approximately Rf = 0.25. Harmine reference standard band is typically present at approximately Rf = 0.34. N,N-dimethyltryptamine (DMT) reference standard band is typically present at approximately Rr = 0.44.

Acceptance Criteria:

The fingerprints obtained with the test solutions, representing Banisteriopsis caapi vine (stem) and Psychotria viridis leaf, are similar to the corresponding reference samples with respect to the phytochemical constituent profile (fingerprint) of the botanical reference materials. Fingerprints for test samples of Psychotria viridis leaf show a band corresponding to the reference standard for DMT. Fingerprints for test samples of Banisteriopsis caapi vine (stem) show bands corresponding to the reference standards for Harmine, Harmaline, and THH, and faint bands corresponding to Harmol and Harmalol may also be present. The color and intensity of the bands may or may not vary. Samples of other identity, if present, yield different fingerprints.

References

1. Wagner, Hildebert. Plant Drug Analysis 2nd Edition. Springer, 2009.

2. Alkemist Labs ID # 141768

Additional images may be collected (before or after derivatization) to ensure an optimal fingerprint evaluation of the phytochemical constituent profile



Identification of Ayahuasca (Banisteriopsis caapi vine) and Chacruna (Psychotria viridis

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dimethyltryptamine are essential for system suitability and evaluation.

Critical Control Point: This Document is the Confidential Property of Appropriate voucher reference samples, if available, or equivalent for Banisteriopsis caapi vine and Psychotria viridis leaf are

Typical Chromatograms

HPTLC **Botanical Test Method**

Latin names: 1) Banisteriopsis caapi (Spruce ex Griseb.) Morton [Malpighiacea essential for identification. Reference standards for harmine, harmaline, tetrahydroharmine, harmalol, and N,N-2) Psychotria viridis Ruiz & Pav. [Rubiaceae]

leaf) by HPTLC

Common Name: 1) Ayahuasca, 2) Chacruna Plant Part: 1) vine (stem), 2) leaf Targeted Compounds: 1) β-carbolines, 2) N, N-dimethyltryptamine

Test Sample/Reference Sample Preparation:

Raw Material: Mix approximately 0.3 g powdered raw material with approxim 50°C for 30 minutes, centrifuge and filter through a cotton plugged Pasteur pi

Water extract: Evaporate 2mL water extract at approximately 70°C to dryness (Image 1) UV 254 nm plugged Pasteur pipette, and use filtrate as test sample.

Standard Preparation:

About 0.05 mg Harmine/mL Methanol About 0.05 mg Harmaline/mL Methanol About 0.2 mg Tetrahydroharmine (THH)/mL Methanol About 0.05 mg Harmol/mL Methanol About 0.05 mg Harmalol/mL Methanol About 0.1 mg N, N-dimethyltryptamine (DMT)/mL Methanol

Detection Reagent Preparation:

Anisaldehyde Dip Reagent:

170 mL of ice cooled methanol is mixed with 20 mL acetic acid, 10 mL sulfuric Images 1-4: Anisaldehyde Spray Reagent:

8.5 mL of ice cooled methanol is mixed with 1 mL acetic acid, 0.5 mL sulfuric a with increasing Rr) (1µL) Reference Standards Volumes used in preparation may be increased or decreased, so long as the pr. Lane 2: Banisteriopsis caapi vine KD20099BD (1 uL) BRM

Stationary Phase:

HPTLC plates 20x10 cm Si 60 F254 (Merck) Part # 1.05642.0001 or equivalent

Sample Application:

Application is performed using the CAMAG Linomat 5 or equivalent. After eac Lane 7: Psychotria viridis leaf MK13001BD (1 µL) BRM methanol: formic acid, and then twice with methanol before the next applicat reference material extract solutions, and 1 µL reference standard as 6 mm bal System Suitability: edge of plate. Injection volume may need adjustment; this is to ensure optima Harmol and Harmalol reference standard bands are typically present at approximately Rr = 0.04. accounting for natural variation of the sample material.

Mobile Phase:

Chloroform/Acetone/Diethylamine (5/4/1; v/v/v) Mobile phase is prepared fresh. Use HPLC grade where available.

Development:

20x10 cm Twin Trough Chamber (CAMAG or equivalent) saturated with mobil paper. Develop to 70 mm from the bottom of the plate and dry the plate for 1 Temperature and humidity at ambient conditions if using manual developmer CAMAG ADC2.

Detection:

- Images taken with CAMAG TUC Visualitier.
- 1. UV 254 nm (Image 1)
- 2. UV 366 nm (Image 2)
- 3. Derivatize with anisaldehyde reagent heated at 100°C for 2 minutes.



Lane 1: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT, Lane 3: Banisteriopsis caapi vine KD13702KT1 (1 µL) BRM Lane 4: Banisteriopsis caapi vine 20205YCV (1 uL) TS Lane 5: Banisteriopsis caapi water extract 20210LSN (1 µL) TS Lane 6: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT, with increasing R_f) (1µL) Reference Standards

Tetrahydroharmine (THH) reference standard band is typically present at approximately $R_f = 0.21$. Harmaline reference standard band is typically present at approximately $R_f = 0.25$. Harmine reference standard band is typically present at approximately Rr = 0.34. N,N-dimethyltryptamine (DMT) reference standard band is typically present at approximately Ri = 0.44.

Acceptance Criteria:

The fingerprints obtained with the test solutions, representing Banisteriopsis caapi vine (stem) and Psychotria viridis leaf, are similar to the corresponding reference samples with respect to the phytochemical constituent profile (fingerprint) of the botanical reference materials. Fingerprints for test samples of Psychotria viridis leaf show a band corresponding to the reference standard for DMT. Fingerprints for test samples of Banisteriopsis caapi vine (stem) show bands corresponding to the reference standards for Harmine, Harmaline, and THH, and faint bands corresponding to Harmol and Harmalol may also be present. The color and intensity of the bands may or may not vary. Samples of other identity, if present, yield different fingerprints.

References:

- Wagner, Hildebert. Plant Drug Analysis 2nd Edition. Springer, 2009.
- 2. Alkemist Labs ID # 141768
- Derivatize with anisaldehyde reagent heated at 100°C for 2 minutes. UV 366 nm (Image 4)

Additional images may be collected (before or after derivatization) to ensure an optimal fingerprint evaluation of the phytochemical constituent profile.



(Image 2) UV 366 nm



(Image 4) Derivatized with Anisaldehyde Reagent. UV 366 nm

Lane 8: Psychotria viridis leaf 20205XJO (1 µL) TS Lane 9: Psychotria ligustrifolia leaf GV13702KT (1 µL) BRM Lane 10: Psychotria nervosa leaf GW13702KT (1 µL) BRM Lane 11: Psychotria punctata leaf GX13702KT (1 µL) BRM Lane 12: Psychotria alba leaf GY13702KT (1 µL) BRM Lane 13: Diploptervis cabrerana leaf DW13702KT (1 µL) BRM Lane 14: Mimosa hostilis leaf HZ13702KT (1 µL) BRM Lane 15: (Harmol, Harmalol, THH, Harmaline, Harmine, DMT, with increasing Rf) (1µL) Reference Standards

Alkemist Labs: Certificate of Analysis, Macroscopy

B. caapi \rightarrow

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Certificate Issued To: Alkemist Labs USA











3

Company Name:	Alkemist Labs
Title:	Banisteriopsis caapi
Plant Part:	stem/vine
Sample Received:	7/23/2020
Sample Description:	Clear Reclosable Plastic Bag
Form of Botanical:	fresh plant specimen
Appearance:	(1) Brown Whole Bark
Lot :	Lot 3, # 81
Sample :	20205YCV 2
Lafin Name:	Banisteriopsis caapi
Reference Sample :	KD13702KT1, KD200998D; Banisteriopsis caapi (Spruce ex Griseb.) [Malpighiaceae] authenficated by macroscopic, microscopic &/or TLC studies according to the reference sources cited below; held at Alkemist Labs, Garden Growe CA
Analyst:	E. Sudberg & P. Fast
Magnification:	(1) Macroscopy
Sample Findings:	(1) whole stem segments showing spiraling longitudinal ridges formed by lobed xylem
Magnitication:	(2) Macroscopy
sample rindings:	(2) lobes of the secondary xylem in cross section
Magnitication:	(3) Macroscopy
Sample hindings:	(3) bark with numerous small, round lenticels
Reference Source:	Flora Neotropica Vol. 30, NY Botanical Garden, Banisteriopsis, Diploptens (Malpighiaceae) (Feb. 18, 1982), pp. 1- 237; Sonia, J. "Wood anatomical distinction of Ianas (Banisteriopsis caapi) used in ritualistic ceremonial of ayahuasca" (UnB).
	MIC-SOP-54-04, MIC-SOP-54-05, MIC-SOP-54-06, MIC-SOP-510-07

Comments & Conclusions: This sample has characteristics of Banisteriopsis caapi (Spruce ex Griseb.) [Malpighiaceae] stem based on comparison with authenticated reference samples and the characteristics described in the references cited above. The characteristic structures identified in this sample are the whole stem segments showing sprinting longitudinal ridges formed by lobed sylem, seen in image (1) above. In image (2) we see the lobes of the secondary xylem in cross section seen in micrograph. In image (2) we see the bark with numerous small, round tertricels. This test sample, Banisteriopsis caapi (Spruce ex Griseb.) (Malpighiaceae] (Lot 3, # 81), has characteristics of Banisteriopsis caapi stem.

NOTE: The presence of soluble excipients and other plant species material was not detected in this test sample.

Analyzed by: Patrick Fast Examined, Reviewed & Authorized by: Sidney Sudberg, CSO

Report Date: 8/31/2020

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<u>Alkemist Labs:</u> <u>Certificate of Analysis,</u> <u>Microscopy</u>

B. caapi →

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Certificate Issued To: Alkemist Labs USA



Alkemist Labs 12661 Hoover Street Garden Grove, CA 92841 714-754-HERB (4372) sales@alkemist.com

Work performed at:



Certificate of Analysis: Banisteriopsis caapi (Lot 3, # 81) Microscopy with Digital Photo-Documentation



3

Jompany Name:	Arkemist Lobs
iffe:	Banisteriopsis caapi
Plant Part:	stem/vine
ample Received:	7/23/2020
ample Description:	Clear Reclosable Plasfic Bag
form of Botanical:	fresh plant specimen
Appearance:	(1) Brown Whole Bark
.ot :	Lot 3, # 81
ample :	20205YCV 2
afin Name:	Banisteriopsis caapi (Spruce ex Griseb.) [Malpighiaceae]
Reference Sample :	KD13702KT1, KD20099BD Banisteriopsis caapi (Spruce ex Griseb.) [Malpighiaceae] authenficated by macroscopic, microscopic &/or TLC studies according to the reference sources cited below; held at Alkemist Labs, Garden
Inshut	E Sudhan I D Eart
Aganification:	(2) 200X
Chemical Reagents:	(2) acidified chloral bydrate alycerol solution
ample Findinas:	(2) fibres with sheath of cells containing pism crystals
Aganification:	(3) 400X
Chemical Reagents:	(3) acidified chloral hydrate glycerol solution
ample Findings:	(3) groups of irregularly shaped, thick-walled, pitted sclereids with blue coloration
Reference Source:	Flora Neotropica Vol. 30, NY Botanical Garden, Banisteriopsis, Diplopterys (Malpighiaceae) (Feb. 18, 1982), pp. 1-
	237; Sonsin, J. "Wood anatomical distinction of lianas (Banisteriopsis caapi) used in ritualistic ceremonial of ayahuasca" (UnB).
	MIC-SOP-54-04, MIC-SOP-54-05, MIC-SOP-54-06, MIC-SOP-510-07

<u>Comments & Conclusions</u>: This sample has characteristics of Banisteriopsis caapi (Spruce ex Griseb.) [Malpighiaceae] stem based on comparison with authenticated reference samples and the characteristics described in the references cited above. The characteristic cellular structures identified in this sample are the fibers with sheath for cells containing prime crystals seen in micrograph (2) above. In micrograph (3) we see the groups of inegularly shaped, thick-walled, pitted sciencids with blue coloration. This test sample, Banisterlopsis caapi (Lot 3, # 81), has characteristics of Banisterlopsis caapi (Spruce ex Griseb.) [Malpiphiaceae] stem. NOIT: the prevence of stable sciptions and other parts in the transite.

Analyzed by: Patrick Fast Examined, Reviewed & Authorized by: Sidney Sudberg, CSO

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Report Date: 8/31/2020

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Alkemist Labs: Certificate of Analysis, HPLC

B. caapi \rightarrow

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CERTIFICATE OF ANALYSIS



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eport Issued To:	Alkemist Labs	
	USA	

Sample Name: Banisteriopsis caapi Description: Fresh plant specimen; Brown Whole Bark Lot #: Lot 3. # 81 AL #: 20205YCV_3 Analysis ID: 143065 Received: 07/23/20

Determination of DMT and B-Carbolines content by HPLC PDA



Analysis Date : 09/25/20 Analyzed By: T French

Authorized By: Kirtal Chopra Laboratory Manager

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11/15/2021

Alkemist Labs: Certificate of Analysis, Macroscopy

P. viridis \rightarrow

USA Gorden Grove, CA 92041 71 4-7 54-HERB (4372) sales@akemist.com Certificate of Analysis: Psychotria viridis (Lot 3, A) Microscopy with Digital Photo-Documentation 3 2 Company Name: Akemisi Lobs Psycholria virida Tiller Plani Pari: Leaf Sample Received. 7/23/2020 Clear Reclosable Maslic Baa Sample Description: Form of Boltanical: fesh plant specimen Appearance: Green Leaves Lol: Lol3, A Somole : 20205XUO_2 Psycholria virids [Rubiaceae] Lolin Name: Reference Sample : MK13001BD Psycholria viridis (Rubiaceae) authenticated by macroscopic, microscopic &/or TLC studies according. to the reference sources cited below; held at Akemis Labs, Garden Grove, CA. Analysi: E.Sudberg & P. Fasl Magnification: (I) Macroscopy Sample Findings: (1) leaves eliptic, often widest above the middle, apex acute, base cureate, margins straight, reaching near or all The base of the periole, adaptial surfaces Magnification: (2) Macroscopy Sample Findings (2) leaves elliptic, often widest above the middle, apex acute, base cureate, margins straight, reaching near or at The base of the periole, abasial surface for Left justified leaf, only

Magnification: (3) Macroscopy Sample Finding: (3) abaciat surface of lead with for solae along the primary vein ReferenceSource: Blackledge, R., Taylor, C., "Psychotria Virids - A Botanical source of Dimethylisystamine (DMI), Kowalcask, A., "dentification Challenges in Examination of CommercialPlant Material of Psychotria virids". Acto Polonica Pharmacellica MC-SOP-S404, MIC-SOP-S405, MIC-SOP-S406, MIC-SOP-S10-07

Comments & Conclusions: This sample has characteristics of Psychotria virids (Rubiaceae) leaf based on comparison with an authenticate deviewince sample and the characteristics described in the references chied based. The characteristics inductors identified in this sample are the eliptic feature, of environmental above the middle, apex acute, base contracte, margins shappit, reaching near or at the base of the petitios seen in images (1) and (2) above, the mage (2) we see the abaxist surface of kar with fovestae above the primary vein. This test sample, hystotria virials (cot), A), has characteristics of Psychotria virials (fublicaceae) leaf. With fovestae above, for each model is escated the score.

Analyzed by: Pahick Fail Examine d, Reviewed & Authorized by: Sidney Sudberg, CSO

Certificate Issued To:

Alkemist Labs

Report Date: 8/31/2020

Work performed at:

Alkemist Labs

12/41 Hoover Street

This report applies to he sample investigated and in an accessibly indicative of the quality according of apparently latitical or initiagropatics. This report floating is a sample of the parties includes and the part of the sample of of the



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Alkemist Labs: Certificate of Analysis, Microscopy

P. viridis \rightarrow

Company Name: Alkemist Labs Psychotia viridis PlantPart: Leaf 7/23/2020 Sample Received: Sample Description: Clear Reclosable Plastic Bag Form of Botanical: fesh plantspecimen Green Leaves Appearance: Lot 3, A 2020.5XJO 2 Sample : Lafin Name: Psychotia viridis (Rubiacea e) Reference Sample : Mit 3001BD Psychatria viridis [Rubia ce ae] authentica ted by macroscopic, microscopic &/ar TLC studies according to the reference sources cited below held at Akemist Labs, Garden Grove, CA. E. Sudberg & P. Fast Analyst Magnification: (1) 400X Chemical Reagents: () acidified chloral hydrate glycerol solution Sample Findings: () epidermal cels with motted surface and thickened wals (2) 200X Magnification: Chemical Reagents: 2) acidified chloral hydrate alværol solution Sample Findings: multicellular, uniseria te covering trichomes with pointed apical cell (some with orange cell contents) (3) 200X Magnification: Chemical Reagents: acidified chloral hydrate glycerol solution. (3) leaf fissue with oil drop lets and crystal raphidles. Sample Findings: Blackledge, R., Taylor, C. "Psychotia Viridis- A Botanical source of Dimethyltyptamine (DIVT); Kowalczuk, A. Reference Source: "Identification Challenges in Examinating of Commercial Plant Material of Psychotria viridis". Acta Polonia e Pharmace utica MC80P-5404, MC-80P-54-05, MC-80P-54-06, MC-80P-510-07

Certificate of Analysis: Psychotria viridis (Lot 3, A) Nicroscopy with Digital Photo-Documentation 2

Comments & Conclusions: This sample has characteristics of Psychotria viridis (Rubiaceae) leaf based on comparison with an authenticated reference sample and the characteristics described in the references cited above. The characteristic cellular structures identified in this sample are the epidermal cells with mottled surface and thickened walk seen in micrograph (1) above. In micrograph (2) we see the multicellular, unseriate covering tichames with pointed apical cell (some with arange cell contents). In micrograph (3) we see the leaf issue with oil droplets and crystal raphides. This test sample, Psychotria viridis (Lot 3, A), has characteristics of Psychotria viridis [Rubia ceae] leof.

NOT: The presence of soluble excisients and other plant species material was not detected in this lest somely.

Analyzed by: Patrick Fast copyright 2021 by Alkemist Labs, all rights reserved Examined, Reviewed & Authorized by: Sidney Sudberg, CSO

Report Date: 8/31/2020

Work performed at: Alkemist Labs

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14



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Alkemist Labs

USA

Title:

Lot :

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Alkemist Labs: Certificate of Analysis, HPLC

P. viridis \rightarrow

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Report Issued To: Alkemist Labs

S4-4372 | r. 714-668-9972 | www.aukemist.com
Sample Name: Psychotria viridis
Description: Fresh plant specimen; Green Leaves
Leaves

ALKEMIST LABS

Lot #: Lot 3, A AL #: 20205XJO_3 Analysis ID: 143060 Received: 07/23/20

Determination of DMT and β-Carbolines content by HPLC PDA



Ret. Time (min)	Compound Name	Prep 1 (%)	Prep 2 (%)	Average (%)	Specification	Result
9.3	N,N-Dimethyltryptamine	0.840	0.814	0.827	Report Only	N/A
10.9	Harmalol	ND	ND	ND	Report Only	N/A
12.4	Harmol	ND	ND	ND	Report Only	N/A
13.7	Tetrahydroharmine	ND	ND	ND	Report Only	N/A
19.8	Harmaline	ND	ND	ND	Report Only	N/A
18.7	Harmine	ND	ND	ND	Report Only	N/A

Chromatographic Con	ditions:
Method:	Determination of Tryptamines and β-Carbolines in Ayahuasca Beverage Consumed During Brazilian
	Religious Ceremonies, Santos Et Al., Journal of AOAC International Vol. 100, No. 3, 2017
Column:	AP281 Zorbax Eclispe Plus C8 5µm (150x4.6)
Temperature:	25°C
Flow Rate:	1.5 mL/min
Injection Volume:	20 µL
UV Detection:	246 nm, 278 nm, 291 nm, 372 nm
Mobile Phase:	0.1% Formic Acid in Water
	0.1% Formic Acid in Methanol
HPLC Instrument:	Alliance_3

Sample Preparation:

Ground sample to a fine powder. Transferred about 100 mg of sample to a 15 mL centrifuge tube and added 10 mL of methanol. Vortexed for 30 seconds and sonicated 30 minutes at room temperature. Let cool and transferred supernatant to a 50 mL volumetric flask. Repeated extraction two additional times for a total of three times. Filled volumetric flask to volume with methanol. Mixed well and filtered through a 0.2 µm PTFE syringe filter. Diluted filtrate 1:10 in water.

Report Summary:

This "Psychotria viridis" test sample contains an average of 0.83% DMT.
N/A
LC149 p. 147

Analysis Date : 09/25/20 Analyzed By: T French

Authorized By: Kirtal Chopra, Laboratory Manager

This report applies to the sample investigated and is not necessarily indicative of the quality or condition of apparently identical or similar products. This report is for the exclusive use of the party who requested the report and not for public assemination or use by third parties, including for promotional purposes, without the prior written permission of Alkemist Labs. This report provides technical results for a specific sample and the report shall not be altered, modified, supplemented or abstracted in any manner. Any violation of these conditions renders the report and its results void 0 2018 Alkemist Labs All Hights Reserved 11/15/2021 15









Psychotria viridis (Lanes 4, 10), water extracts: Characterization/Quantitation



Ayahuasca (weight) (Lanes 5, 11), water extracts: Characterization/Quantitation







Ayahuasca (volume) (Lanes 6, 12), water extracts: Characterization/Quantitation







DMT: water extracts, Quantitation, Scanning Densitometry



Sample	R _f	Concentration	Amount on Plate	Amount per Sample
1) <i>B. caapi</i> YCV (S1)	N/A	N/A	N/A	N/A
2) <i>B. caapi</i> YCV (S2)	N/A	N/A	N/A	N/A
3) P. viridis XJO (S1)	0.445	1.152 mg/mL	576.2 ng	2.305 mg in 1000.0 mg
4) P. viridis XJO (S2)	0.455	1.326 mg/mL	663.2 ng	2.653 mg in 1000.3 mg
5) YCV + XJO (weight) (S1)	0.447	0.816 mg/mL	816.3 ng	3.266 mg in 1001.4 mg
6) YCV + XJO (weight) (S2)	0.455	0.777 mg/mL	777.7 ng	3.110 mg in 1001.6 mg
7) YCV + XJO (volume) (S1)	0.448	0.597 mg/mL	597.5 ng	2.390 mg in 1000.0 mg
8) YCV + XJO (volume) (S2)	0.457	0.653 mg/mL	653.0 ng	2.612 mg in 1000.3 mg

Harmine: water extracts, Quantitation, Scanning Densitometry



Sample	ole R _f Concentration		Amount on Plate	Amount per Sample
1) <i>B. caapi</i> YCV (S1)	0.338	0.333 mg/mL	166.4 ng	0.666 mg in 1001.6 mg
2) B. caapi YCV (S2)	0.348	0.278 mg/mL	139.1 ng	0.556 mg in 1001.6 mg
3) P. viridis XJO (S1)	viridis XJO (S1) N/A N/A N/A		N/A	
4) P. viridis XJO (S2)	N/A	N/A	N/A	N/A
5) YCV + XJO (weight) (S1)	0.340	0.110 mg/mL	109.3 ng	0.219 mg in 1001.2 mg
6) YCV + XJO (weight) (S2)	0.348	0.123 mg/mL	122.8 ng	0.246 mg in 1001.6 mg
7) YCV + XJO (volume) (S1)	0.342	0.159 mg/mL	159.2 ng	0.637 mg in 1001.6 mg
8) YCV + XJO (volume) (S2)	0.352	0.141 mg/mL	140.8 ng	0.563 mg in 1001.6 mg

METHANOL EXTRACTION: QUANTITATIVE DATA



DMT: methanol extracts, Quantitation, Scanning Densitometry



Sample	R _f	Concentration	Amount on Plate	Amount per Sample
1) <i>B. caapi</i> YCV (S1)	N/A	N/A	N/A	N/A
2) <i>B. caapi</i> YCV (S2)	N/A	N/A	N/A	N/A
3) P. viridis XJO (S1)	0.447	0.566 mg/mL	283.1 ng	5.662 mg in 1004.8 mg
4) P. viridis XJO (S2)	0.450	0.635 mg/mL	317.5 ng	6.350 mg in 1006.2 mg
5) YCV + XJO (weight) (S1)	0.447	0.326 mg/mL	326.3 ng	6.528 mg in 1006.0 mg
6) YCV + XJO (weight) (S2)	0.450	0.322 mg/mL	321.6 ng	6.432 mg in 1006.8 mg
7) YCV + XJO (volume) (S1)	0.448	0.251 mg/mL	250.6 ng	5.012 mg in 1004.8 mg
8) YCV + XJO (volume) (S2)	0.450	0.299 mg/mL	298.6 ng	5.972 mg in 1006.2 mg

Harmine: methanol extracts, Quantitation, Scanning Densitometry



Sample	R _f	Concentration	Amount on Plate	Amount per Sample
1) <i>B. caapi</i> YCV (S1)	0.347	0.523 mg/mL	261.6 ng	5.232 mg in 1006.4 mg
2) B. caapi YCV (S2)	0.348	0.414 mg/mL	206.7 ng	4.134 mg in 1005.2 mg
3) P. viridis XJO (S1)	N/A	N/A	N/A	N/A
4) P. viridis XJO (S2)	N/A	N/A	N/A	N/A
5) YCV + XJO (weight) (S1)	0.348	0.273 mg/mL	272.6 ng	5.452 mg in 1013.6 mg
6) YCV + XJO (weight) (S2)	0.350	0.249 mg/mL	249.2 ng	4.984 mg in 1007.2 mg
7) YCV + XJO (volume) (S1)	0.348	0.242 mg/mL	242.0 ng	4.840 mg in 1006.4 mg
8) YCV + XJO (volume) (S2)	0.350	0.186 mg/mL	186.3 ng	3.726 mg in 1005.2 mg



Harmine: Water (w/w) % Harmine: Water (v/v) % Harmine: Methanol (w/w) % Harmine: Methanol (v/v) % DMT: Water (w/w) % DMT: Water (v/v) % DMT: Methanol (w/w) % DMT: Methanol (v/v) %



AY256617UBG4_1: Palmers Cocoa Butter Formula lot B6JUL - S2
AY25661/UBG4_1: Palmers Cocoa Butter Formula lot B6JUL - S1
JAY25661/UBG3_1: J5 lot J5 - S2
JAY256617UBG3_1: J5 lot J5 - S1
JAY256617UBG2_1: J3 lot J3 - S2
JAY256617UBG2_1: J3 lot J3 - S1
JAY256617UBG1_1: J2 lot J2 - S2
JAY256617UBG1_1: J2 lot J2 - S1
JAY15717UGB13: 4 Stars_3 10/2016 New Huito - S2
JAY15717UGB13: 4 Stars_3 10/2016 New Huito - S1
JAY15717UGB12: 3 Stars_1 8/2016 Huito - S2
JAY15717UGB12: 3 Stars_1 8/2016 Huito - S1
JAY15717UGB11: 2 Stars_1 08/2015 Gooy - S2
JAY15717UGB11: 2 Stars_1 08/2015 Gooy - S1
JAY15717UGB10: 4 Stars_1 12/2014 New Mixta with Repair - S2
JAY15717UGB10: 4 Stars_1 12/2014 New Mixta with Repair - S1
JAY15717UGB9: 5 stars + 5/2013 Repair DLC - S2
JAY15717UGB9: 5 stars + 5/2013 Repair DLC - S1
IAY15717UGB8: 2 Stara_2 2/2013 H.B. Load Not Much Light - S2
IAY15717UGB8: 2 Stara_2 2/2013 H.B. Load Not Much Light - S1
JAY15717UGB7: 3 Stars 2 2/2012 U de G - S2
JAY15717UGB7: 3 Stars 2 2/2012 U de G - S1
JAY15717UGB6: 3 Stars 2 5/2011 Ceremony #3 - S2
JAY15717UGB6: 3 Stars 2 5/2011 Ceremony #3 - S1
JAY15717UGB5: 4 Stars 2 2/2011 MIXTA - S2
JAY15717UGB5: 4 Stars 2 2/2011 MIXTA - S1
JAY15717UGB4: 1 Star 1 6/2010 KM 24 - S2
JAY15717UGB4: 1 Star 1 6/2010 KM 24 - S1
JAY15717UGB3: 5 Stars 1 6/2009 REFINO - S2
JAY15717UGB3: 5 Stars 1 6/2009 REFINO - S1
JAY15717UGB2: Stars Unknown 1/2007 To Be Tested - S2
JAY15717UGB2: Stars Unknown 1/2007 To Be Tested - S1
JAY15717UGB1: Agua Flores Lot 04/2014 - S2
JAY15717UGB1: Agua Flores Lot 04/2014 - S1

Sample Results (2017)

AP #	DMT	Harmine
	%	%
JAY256617UBG4_1: Palmers Cocoa Butter Formula lot B6JUL - S1	0.5087	0.8031
JAY256617UBG4_1: Palmers Cocoa Butter Formula lot B6JUL - S2	0.5607	0.9277
JAY256617UBG1_1: J2 lot J2 - S1	0.3691	0.8799
JAY256617UBG1_1: J2 lot J2 - S2	0.3621	0.8851
JAY256617UBG2_1: J3 lot J3 - S1	0.3400	1.2775
JAY256617UBG2_1: J3 lot J3 - S2	0.3422	1.3880
JAY256617UBG3_1: J5 lot J5 - S1	0.2789	1.1698
JAY256617UBG3_1: J5 lot J5 - S2	0.2283	0.8098
JAY15717UGB13: 4 Stars_3 10/2016 New Huito - S1	0.4695	0.8449
JAY15717UGB13: 4 Stars_3 10/2016 New Huito - S2	0.5158	0.9263
JAY15717UGB12: 3 Stars_1 8/2016 Huito - S1	0.3620	0.3169
JAY15717UGB12: 3 Stars_1 8/2016 Huito - S2	0.3601	0.3065
JAY15717UGB11: 2 Stars_1 08/2015 Gooy - S1	0.4138	0.5925
JAY15717UGB11: 2 Stars_1 08/2015 Gooy - S2	0.4217	0.5974
JAY15717UGB10: 4 Stars_1 12/2014 New Mixta with Repair - S1	0.5084	0.9495
JAY15717UGB10: 4 Stars_1 12/2014 New Mixta with Repair - S2	0.5521	1.0734
JAY15717UGB9: 5 stars + 5/2013 Repair DLC - S1	0.6162	1.6035
JAY15717UGB9: 5 stars + 5/2013 Repair DLC - S2	0.6446	1.7085
JAY15717UGB8: 2 Stara_2 2/2013 H.B. Load Not Much Light - S1	0.5800	0.5477
JAY15717UGB8: 2 Stara_2 2/2013 H.B. Load Not Much Light - S2	0.5749	0.5191
JAY15717UGB7: 3 Stars_2 2/2012 U de G - S1	0.2995	0.5256
JAY15717UGB7: 3 Stars_2 2/2012 U de G - S2	0.3404	0.6364
JAY15717UGB6: 3 Stars_2 5/2011 Ceremony #3 - S1	0.4917	1.0956
JAY15717UGB6: 3 Stars_2 5/2011 Ceremony #3 - S2	0.4709	0.9784
JAY15717UGB5: 4 Stars_2 2/2011 MIXTA - S1	0.5180	0.8666
JAY15717UGB5: 4 Stars_2 2/2011 MIXTA - S2	0.4952	0.8658
JAY15717UGB4: 1 Star_1 6/2010 KM 24 - S1	0.6979	0.8941
JAY15717UGB4: 1 Star_1 6/2010 KM 24 - S2	0.6588	0.7784
JAY15717UGB3: 5 Stars_1 6/2009 REFINO - S1	0.5800	1.2233
JAY15717UGB3: 5 Stars_1 6/2009 REFINO - S2	0.5632	1.1763
JAY15717UGB2: Stars Unknown 1/2007 To Be Tested - S1	0.3147	0.3914
JAY15717UGB2: Stars Unknown 1/2007 To Be Tested - S2	0.3654	0.6240
JAY15717UGB1: Agua Flores Lot 04/2014 - S1	0.5628	1.0259
JAY15717UGB1: Agua Flores Lot 04/2014 - S2	0.5527	1.0047

Ayahuasca Concentration with C18 Column (2017)

Results & Conclusions:

This is what we found:

- Weight method yielded higher concentrations for both cpds., except in the case of the water extraction of Harmine, which was greater in the volume method by a factor of ~ 2, which may be significant from a quality perspective?
- The Ideal ratio for Harmine:DMT, suggests:
 - Harmine should be 2 ~ 3 times the concentration of NLT ~ 0.5% DMT
- <u>HPTLC is the preferred method to study these questions, due to its'</u> <u>ability to reduce large data sets to be easily interpreted, especially</u> <u>as applied to the variability of Natural Products, as moving targets.</u>

Future Goals:

- Analyze Ayahuasca ingredients before they are processed to get a baseline composition, by HPTLC
- Analyze the Ayahuasca Brews, in- process, from the Jungle
 - Measure the affect of heating time, based on cpd ratios & subjective effects
 - Measure the active cpd content & their ratios.
 - Specifically DMT, Harmine, THH, Harmaline
 - Monitor efficacy, strength, etc., in vivo, so as to
 - Develop a predictable & reliable experience, if possible.

References

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