





ESTABLISHING IDENTIFICATION METHODS FOR ETHNOBOTANICAL MEDICINES

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What a long strange trip it's been. After all these years there is now a growing interest in the quality control of plant medicines. In the "early" days when there wasn't too much in the way of choice of plant medicine available to the consumer, concern for the quality of your herb of choice was minimal. Now, there seems to be a glut of product available and unless you know how to identify or distinguish good quality vs. poor quality by the age-old, time-tested organoleptic methods of taste, smell, and appearance of the plant, then you are dependent upon the reliability of your source or your own bioassay methods. If you are purchasing a plant medicine in any form other than the whole or cut-and-sifted form, then it becomes even more difficult to identify what plant you are buying or if it is mixed with anything other than the plant you want.

LESSER-KNOWN PLANTS

With the introduction of a number of lesser-known plant medicines from other cultures and parts of the world to the United States and Europe in the last two decades, the need for accurate identification is increasing. A plant species is unlike a pure chemical substance in many ways since very precise criteria such as melting point, spectral characteristics, and a chromatographic comparison with reference substances can be used to establish the identity of a single chemical entity. In the case of plant material, the standards for comparison cannot usually be so precise. Ideally the material to be identified should be compared with authentic specimens of good quality, either in the preserved or living state. This not being practicable, one usually has to rely on comparison against a written description and this can be supplemented with some type of picture of the product.

As the number of plant specimens increases or their usage profile broadens, the likelihood of "adulteration" or even substitution increases. Methods to identify a plant are described in monographs for many plants, but with the lesser-known or more novel plants appearing in commerce today, these are generally not available. Monographs for botanicals usually consist of a description of the distinctive and characteristic macroscopic and microscopic features of the plant in question together with simple descriptions of its organolep-

tic characters. Many monographs also include some type of test to ensure that the correct chemical ingredients are present whether via a "wet" chemical test resulting in the formation of a color or precipitate, or by chromatographic examination. Literature searches may provide additional chemical information. In the case of so many of the visionary plants, a single chemical compound is the defining factor for the quality and/or identity of the plant in question.

CATEGORIES OF IDENTIFICATION

The criteria used for the identification can be divided into four major categories:

- 1) Macroscopic appearance
- 2) Organoleptic characters
- 3) Microscopic appearance
- 4) Presence and absence of chemical substances

Very rarely is identification made on the presence of only one feature. More frequently it is a combination of features that are all consistent with the plant in question. In the examples below, we show how these simple but powerful techniques are useful for plant identification and overall quality.

1) Macroscopic appearance is useful when the botanical is in pieces greater than 2 cm in diameter. Those features which can be seen with the naked eye or with a hand lens are used for identification. The macroscopic criteria most frequently used for identification are shape, size, color, and appearance of all surfaces. But, when there is only a powdered sample, the task of identification is more complex and one must rely on other procedures.

2) Organoleptic characteristics can help identify a large number of botanicals by their odor, appearance, texture, and taste. A classic example of the feature of odor would be *Cannabis sativa*.

3) Microscopic appearance brings us to the world of botanical anatomy. Here is where it gets interesting and where we





can make plant identification a fun and artistic science. Many plants have such unique characteristic cellular structures that there is no mistake as to the identification of the plant. An example might be used by making the analogy to the obvious difference between a rose and a tulip or many other plants for that matter, macroscopically. Most of us can make this distinction by its gross appearance and/or smell and even if it were cut up, you would still be able to distinguish them from one another, to an extent. Similarly, there are obvious *microscopic* characteristics that make plants distinguishable to the trained eye just like the rose is to most of us with the naked eye. In the pictures below you will observe the differences between the leaves of *Salvia divinorum* and *Mitragyna speciosa* both microscopically and chromatographically. If we look at the macroscopic features of the two leaves side by side, there would be a clear difference. If the leaves were powdered, you could perform a bioassay to distinguish the two or you could look at them chemically.

4) Chemical constituent profiles or fingerprints of the secondary metabolites of the plant are unique to each plant. Most “wet” tests are not very specific and detect only a certain classes of compounds. The advent of chromatography, particularly high performance thin-layer chromatography (HPTLC), brought a whole new dimension to the use of chemical constituents for botanical identification. The other major techniques are gas chromatography (GC) for the investigation of essentials, and high performance liquid chromatography (HPLC), which can be used for almost any type of constituent but which has the disadvantage of being comparatively costly and less environmentally friendly compared with HPTLC.

COMBINING TECHNIQUES

Some examples of these combined techniques can demonstrate its utility. We recently had the opportunity to analyze, identify and characterize a plant we weren't familiar with, *Mitragyna speciosa* (*kratom*). There were no references for it that we were able to get and we only had some TLC procedures used for identification given to us for analysis. We were given about 14 different samples and only a few of them were considered reliable or authentic. Of the 14, only seven of them were actually the right plant as determined by the pictures that follow. Our first step was to look at the powdered sample for any characteristic cellular structures that could be used for identification.

In Figure 1 and Figure 2 (below) you are looking at the microscopic image of the surface of the leaf from a powdered sample of *Mitragyna speciosa*. The pointed structures are trichomes or hairs on the leaf midrib. The sample used for these pictures was a reliable sample and therefore can be used as a standard for the identification of future samples.

In order to further enhance this process, we ran a series of HPTLC plates to characterize the chemical profiles (Figures 3–6, to the right). We were supplied with some of a chemical reference standard, the alkaloid mitragynine picrate, which is unique to *kratom* that would allow us to further confirm its identification. By use of these “fingerprints” you can get an idea of the predictable nature of the chemical constituents and the presence of the reference standard used to characterize it. Lanes 1–7 are the different samples and lane 8 is the reference standard mitragynine picrate. In Figures #3 and #4 we used the same chromatographic conditions and see

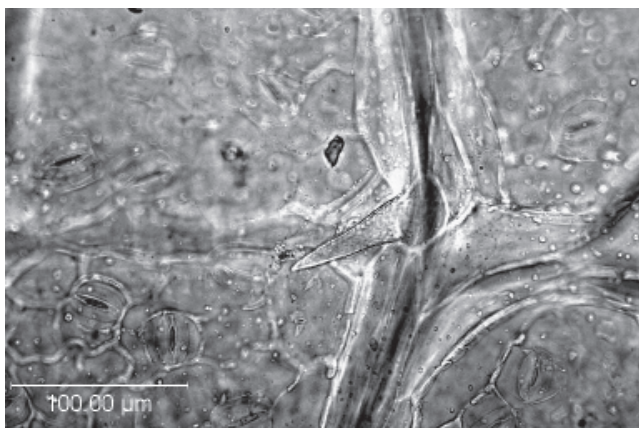


Figure 1: *Mitragyna speciosa*. Sample Findings: unicellular bristle like trichome found on the mid rib showing warted exine. Magnification: 400X. Chemical Reagents: acidified chloral hydrate glycerol solution.

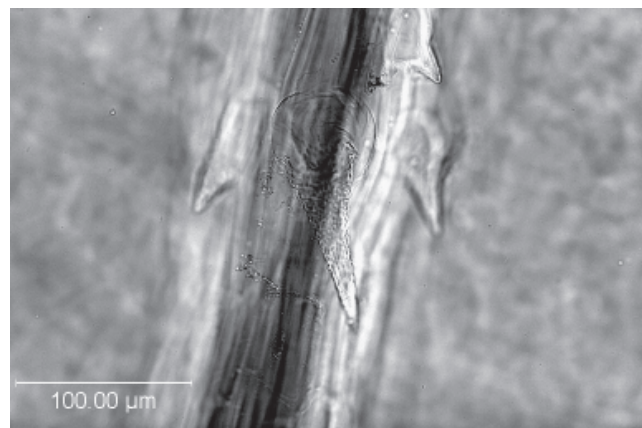


Figure 2: *Mitragyna speciosa*. Sample Findings: unicellular bristle like trichome found on the mid rib showing warted exine. Magnification: 400X. Chemical Reagents: acidified chloral hydrate glycerol solution.



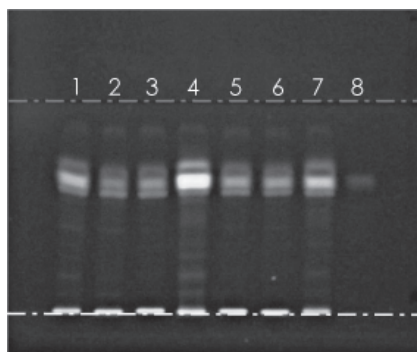


Figure 3: *Mitragyna speciosa*.
Stationary Phase: silica gel 60, F254, 10 x 10 cm HPTLC plates.
Mobile Phase : toluene : ethyl acetate : diethylamine [7.0/2.0/1.0].
Detection: UV 365 nm.

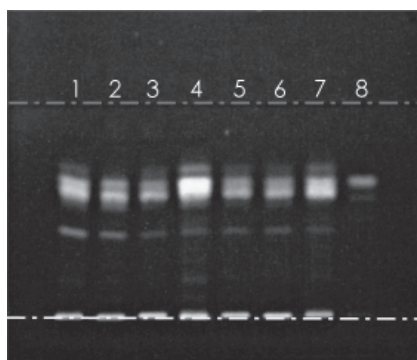


Figure 4: *Mitragyna speciosa*.
Stationary Phase: silica gel 60, F254, 10 x 10 cm HPTLC plates.
Mobile Phase: toluene : ethyl acetate : diethylamine [7.0/2.0/1.0].
Detection: Van Urk's spray reagent à UV 365 nm.

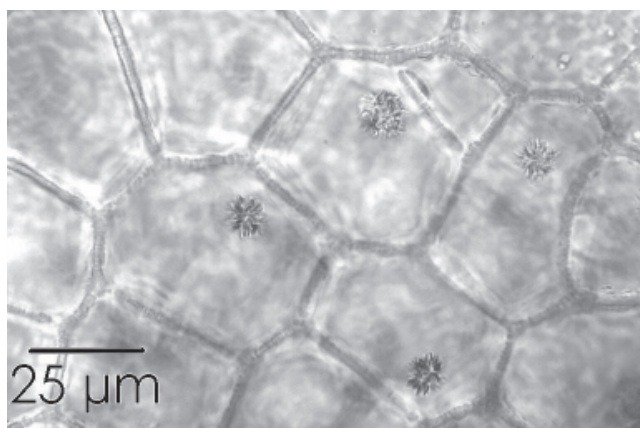


Figure 7: *Psychotria viridis*. Sample Findings: upper epidermis with thick blotchy cuticle. Magnification: 1000X. Chemical Reagents: acidified chloral hydrate glycerol solution.

one fingerprint and when using a different set of chromatographic conditions in Figures #5 and #6 we see a different fingerprint but the patterns are the same, thus adding an element of multi-dimensionality to the characterization of this plant for future reference. If we were to try to draw any conclusions from this study, we could say that by comparison among all these samples, that if we look at lanes 4 in images 3–6, we could easily conclude that this sample of *kratom* has the highest concentration of its constituents including the apparent active principle mitragynine which is in lane 8 in all 4 images. Between the microscopic images of this plant and the HPTLC fingerprints, there would be very little difficulty identifying or distinguishing it from any substitute.

PSYCHOTRIA VIRIDIS

Now for some images of some of the better known plant medicines that we all know and love. In Figure 7 and Figure 8 (below) we see *Psychotria viridis*. These are microscopic images that are not unique to *P. viridis* (as they are common structures in all plants) but they are characteristic of this plant and can be used to exclude other possible plants as substitutes.

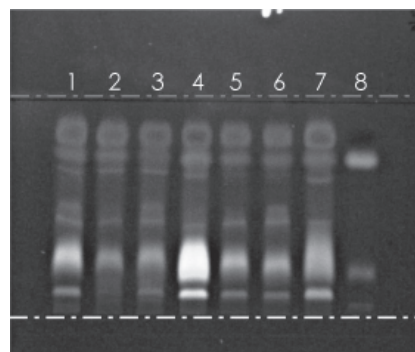


Figure 5: *Mitragyna speciosa*.
Stationary Phase: silica gel 60, F254, 10 x 10 cm HPTLC plates.
Mobile Phase: n-propanol : 1.5% NH4OH [9.0/2.0].
Detection: UV 365 nm.

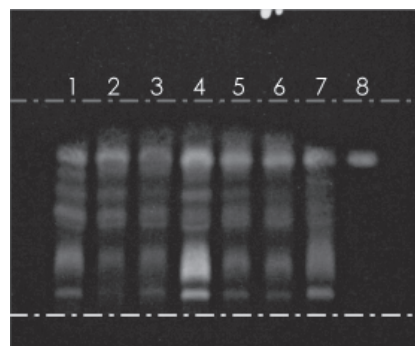


Figure 6: *Mitragyna speciosa*.
Stationary Phase: silica gel 60, F254, 10 x 10 cm HPTLC plates.
Mobile Phase: n-propanol : 1.5% NH4OH [9.0/2.0].
Detection: Van Urk's spray reagent à UV 365 nm.

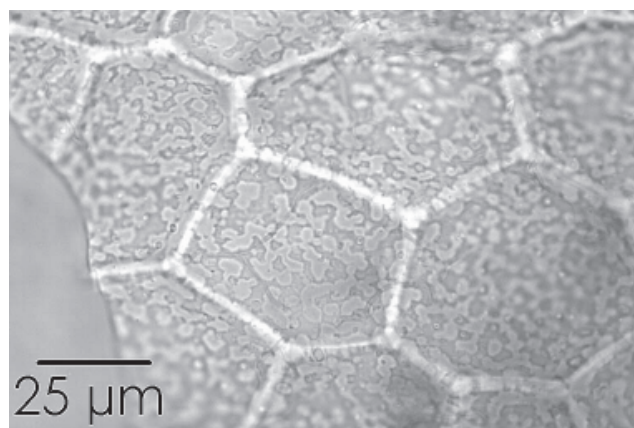


Figure 8: *Psychotria viridis*. Sample Findings: lower epidermis with beaded cell walls & stomate. Magnification: 1000X. Chemical Reagents: acidified chloral hydrate glycerol solution.



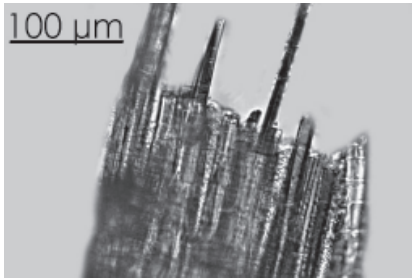


Figure 9: *Banisteriopsis caapi*.
Sample Findings: a bundle of lignified fibers.
Magnification: 400X.
Chemical Reagents: acidified chloral hydrate glycerol solution.

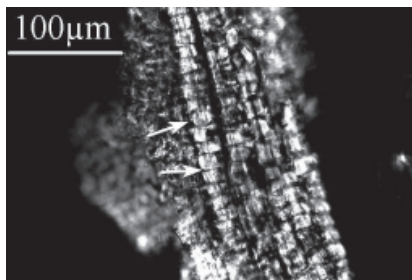


Figure 10: *Banisteriopsis caapi*.
Sample Findings: fibers with inlaid prisms of calcium oxalate.
Magnification: 400X.
Chemical Reagents: acidified chloral hydrate glycerol solution.

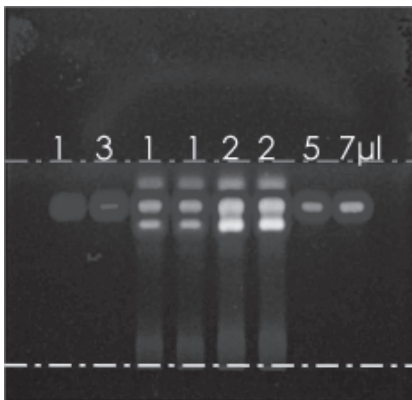


Figure 11: *Banisteriopsis caapi*
Stationary Phase: silica gel 60, F254, 10 x 10 cm HPTLC plates.
Mobile Phase: CH₃OH:CHCl₃:acetic acid [7.5/2.5/1.5].
Detection: UV 365 nm.

BANISTERIOPSIS CAAPI

[Left:] Figures 9 and 10 show some of the microscopic features that help make the identification of this plant. Figure 11 is a HPTLC fingerprint showing the presence of harmine in *B. caapi*.

SALVIA DIVINORUM

[Right:] Figures 12 and 13 are images of cellular structures of the leaf surface showing a top view of one of the glandular hairs and the characteristic trichomes. Figure 14 shows a typical HPTLC profile for *S. divinorum* in the 4 lanes in the middle of the picture with the reference standard salvinorin A in the first and last two lanes with the volumes used to create those bands listed on top of the lanes.

Microscopic pictures in combination with the HPTLC fingerprints are an important tool in Pharmacognosy and have been used for many years for quality control in and for herbs of commerce. Although most of the plants pictured are unlikely to be considered herbs major of commerce in the near future, should the question arise as to the identity of some entheobotanical, whether for personal or commercial purposes, it is reassuring to know that there is a way to monitor the quality of these plant medicines. It is our goal to eventually produce a compendium or reference library of as many of the psychoactive ethnobotanicals as possible in a manual of identification using macroscopy/microscopy and HPTLC. A project of this sort will require that we first have vouchered or authenticated samples of any plants we analyze. If there are any interested parties who would like to participate in a project of this sort can contribute vouchered specimens of unusual ethnobotanical plants, please contact us through our web site at www.alkemist.com. ☉

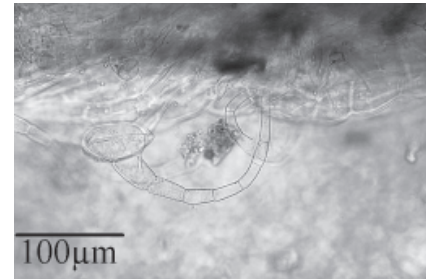


Figure 12: *Salvia divinorum*.
Sample Findings: glandular trichome showing four individual cells.
Magnification: 400X.
Chemical Reagents: acidified chloral hydrate glycerol solution.

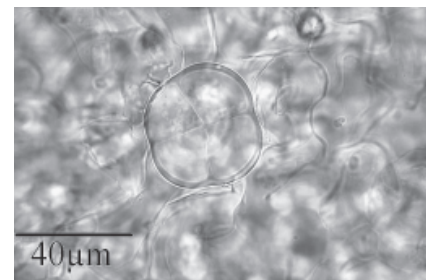


Figure 13: *Salvia divinorum*.
Sample Findings: large multicellular trichome with warty exine.
Magnification: 1000X.
Chemical Reagents: acidified chloral hydrate glycerol solution.

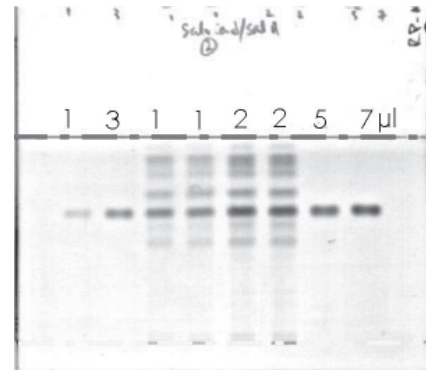


Figure 14: *Salvia divinorum*.
Stationary Phase: RP-18W/UV254, 10 x 10 cm HPTLC plates.
Mobile Phase: ethyl acetate : hexanes [5.0/5.0].
Detection: Vanillin – H₃PO₄ spray reagent.

