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Rapid detection by direct analysis in real time-mass spectrometry (DART-MS) of psychoactive plant drugs of abuse: The case of *Mitragyna speciosa* aka "Kratom"

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ABSTRACT

Mitragyna speciosa, also known commonly as "Kratom" or "Ketum", is a plant with psychoactive properties that have been attributed to the presence of various indole alkaloids such as mitragynine and 7-hydroxymitragynine. *M. speciosa* use is gaining popularity internationally as a natural and legal alternative to narcotics. As a drug of abuse, its detection and identification are not straightforward, since *M. speciosa* plant material is not particularly distinctive. Here, we show that direct analysis in real time-mass spectrometry (DART-MS) can be used not only to rapidly identify *M. speciosa* plant material and distinguish it from other plants, but also to distinguish between *M. speciosa* plant varieties, based on differences between their chemical profiles. The method is rapid and the analysis expeditious. Plant material such as that found at a crime scene can be analyzed directly with no sample pre-preparation steps. Furthermore, we show that the basis set of principal components that permit characterization of the plant material can be used to positively identify *M. speciosa*.

1. Introduction

In response to the expansion of controlled substances lists and the institution of analog drug laws, the past decade has witnessed the ascendance of "legal" psychoactive substances, so labeled because although purported to have mind altering characteristics, their manufacture, possession, and use remain unscheduled in many countries. Trade in whole plant legal psychotropics presents numerous perceived advantages to the drug manufacturer and user, not the least of which are the ease of sale and distribution through the internet, and the fact that the evidentiary value of such plants or plant products is limited due to the challenge of identifying them. Thus, unlike the cannabis plant that has characteristic well-recognized foliage and easily identified scheduled alkaloids, numerous other plants with physical characteristics that are not widely known and which contain unscheduled psychoactive substances, are available and exploited. This allows both the user and manufacturer to enjoy freedom from prosecution, while at the same time, exposing both to the potentially life-threatening consequences of unregulated exposure to dangerous and toxic active ingredients. The challenges this imposes on law enforcement agencies and crime laboratories are obvious, as the availability of rapid and facile testing protocols for identification of such substances or the plants from which they are derived can be limited or non-existent.

An example of one such plant is *Mitragyna speciosa*. Known colloquially as "Kratom", it is endemic to tropical and sub-tropical regions of Southeast Asia and Africa. It has been used in traditional medicine to treat intestinal disorders, muscle pain, coughing, and diarrhea, as well as for its psychotropic effects [1–5]. Preparations of the plant's aerial parts have been shown to have analgesic, euphoric, and anti-depressant effects [4]. Thai and Malaysian laborers and farmers have been reported to use Kratom as a stimulant in order to provide stamina and relief from sore muscles [6]. Although planting *M. speciosa* in Thailand has been illegal since 1943 and ingestion of the plant was outlawed in 1979, Kratom remains a popular drug in Thailand [4].

Even in those countries where it is currently unscheduled, Kratom is of particular interest to the forensics community because multiple poisonings and fatalities have been associated with its use [7–9]. In these cases, testing for the presence of Kratom was prompted by either self-reporting by the user [7], residual Kratom found in the surroundings of the deceased, or a history of

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past opioid use [8]. As there are no published toxicity thresholds for the psychoactive components of Kratom, notably mitragynine, it is often difficult to ascertain the extent to which ingested Kratom contributes to poisonings or fatalities [8].

Although the plant is often ingested alone, Kratom has also been found to be a component of herbal smoking blends that have become popular in the past decade. In Germany and Sweden, products sold under the name "Krypton" were actually enhanced Kratom preparations that also contained both caffeine and O-desmethyltramadol [9]. In 2009 alone, nine fatal overdoses attributed to the use of Krypton were reported [4,10].

Even though Kratom is unscheduled in the United States (US), the US Drug Enforcement Administration has classified it as a "Drug of Concern" and issued a bulletin on Kratom in January 2013 [11]. Kratom use in the US has increased in recent years. Whereas only one case was reported in 2010, that number increased to 44 in 2011, and then it further increased to 81 cases during the first six months of 2012 [1]. Mitragynine and other *M. speciosa* alkaloids, as well as their metabolites, are often not part of routine drug screens. Although testing protocols such as an ELISA assay [12], as well as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFL)-based identification systems [13,14] have been developed, the adoption of such protocols by forensics labs may be slow due to the significant escalation in costs that adoption of these methods may impose. Nevertheless, other testing methods specific for Kratom have recently been reported, reflecting the increasing importance of the emerging problem of Kratom abuse. These methods include HPLC– or LC–MS/MS analysis of Kratom extracts [6,15–20].

The psychotropic activity of Kratom has been attributed to various indole alkaloids whose presence and concentration vary as a function of where the plant is grown [11]. Mitragynine and its stereoisomers mitraciliatine, speciogynine, and speciociliatine, as well as the related compound paynantheine, are abundant alkaloids present in the leaves of *M. speciosa* [21] and these species are reported to be agonists of κ and μ-subtype opioid receptors (Fig. 1) [2,3,5,11,15,20,22,23]. In terms of opioid receptor agonism, mitragynine is comparable with morphine. However, 7-hydroxymitragynine has been reported to exhibit 13–17 times the agonism of morphine toward opioid receptors [24–26]. These studies have also shown that 7-hydroxymitragynine induces morphine-like tolerance and withdrawal in rats [25,26].

Anecdotal reports describe symptoms of withdrawal in humans that include intense craving, chronic fatigue, insomnia and sudden nerve pain [27,28]. As mitragynine and 7-hydroxymitragynine have not been found in any other plant [2,5], including those in the Rubiaceae family of which *M. speciosa* is a member, both compounds could serve as possible chemical markers and/or chemotaxonomic identifiers of Kratom.

Currently the evidentiary value of Kratom is limited, as plant material found at a crime scene may be difficult to identify. Evidence could be found as live plants, dried leaves, or a powdered leaf

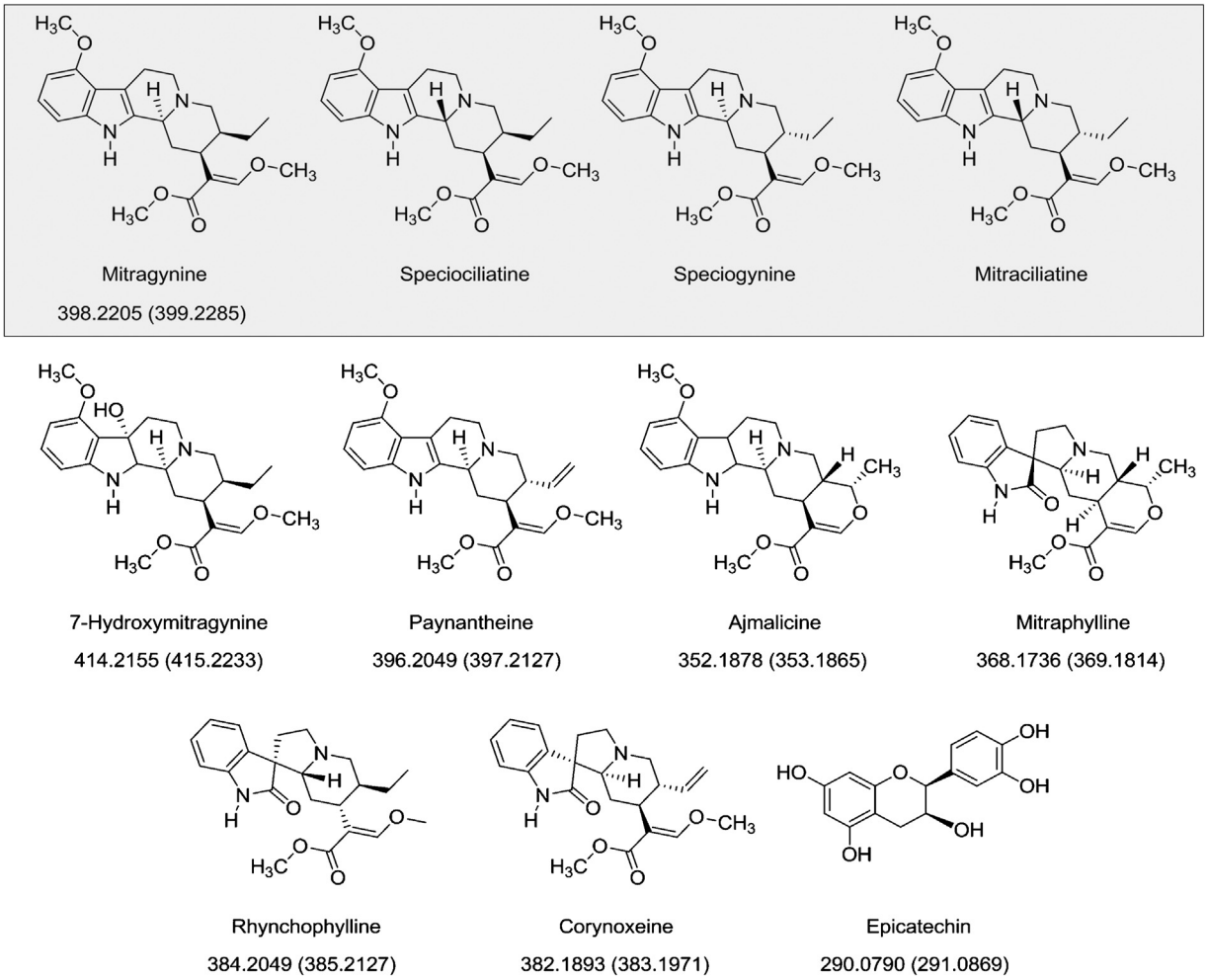

Fig. 1. The names and structures of alkaloids and a flavonoid reported to be found in *M. speciosa*. Their calculated masses as well as the calculated masses of their protonated forms (in parentheses) are shown. Molecules in the shaded area are stereoisomers of one another and therefore have the same molecular weights.