Journal of Forensic Research
ISSN: 2157-7145

Drake et al., J Forensic Res 2019, 10:2

Review Article Open Access

The Structural Complexities of *Cannabis sativa* L. and Profiling Techniques for Geographic Source Determination

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Received date: June 03, 2019; **Accepted date:** June 17, 2019; **Published date:** June 24, 2019

Abstract

Marijuana is the most widespread abused, trafficked, and consumed drug in America. Although according to federal law, the use, consumption, and distribution of marijuana are illegal, approximately two-thirds of the State Governments in the U.S. and the District of Columbia have legalized marijuana for either medical or recreational purposes. Due to the passing of new controversial marijuana legislation at the state levels of government, which started with the passing of Amendments 64 and Initiative 502, respectively, in Colorado and Washington in 2012, the federal government is faced with many challenges associated with enforcing and preventing the illegal diversion of marijuana and marijuana products. Although state legislation may be passed to legalize marijuana for particular jurisdictions, under the Controlled Substances Act (CSA), distribution and possession of marijuana is still illegal on a federal level, and marijuana is classified as a Schedule I controlled substance. Controlling the distribution and trafficking of "legalized marijuana" to minors and improper channels is also a major challenge that law enforcement now faces. However, to date, no extensive registry (chemical nor DNA) or tracking system has been implemented to prevent the diversion of these products to neighbouring states, where the consumption or distribution of these materials remain illegal on the state level. Although the legal requirement in most countries in the identification of marijuana is limited to the confirmation of the presence of cannabinoids (THC) and cystolithic hairs on leaves, several techniques have been published and implemented to investigate the origin of marijuana samples (including palynology, DNA profiling, chemical profiling and isotopic analysis.

In this paper, the chemical complexities of *C. sativa* L. are presented along with an overview of profiling techniques, which have been proposed for geographical source determination of marijuana. This may provide the forensic community with insight regarding the use of different profiling methods as potential tools in assessing the identification and origin of cannabis samples to support interdiction efforts. Additionally, this study can provide forensic practitioners with relevant information about specificity, discriminatory power, scope, and limitations of different profiling methods for the determination of source origin of marijuana samples. Chemical profiling is proposed as an efficient, robust, and reliable method, which may be a powerful tool for identifying the source of seized Marijuana evidence. Ultimately, chemical profiling techniques may offer forensic laboratories a path forward in establishing links between grow operations, trafficking routes and supply chains, which can ultimately assist in interdiction efforts.

Keywords: *Cannabis sativa* L.; Chemical profiling techniques; Cannabinoids; Terpenes; Geographic source determination

Introduction

Cannabis, one of only two genuses belonging to the Cannabacea family, is a dioeciously annual plant originating from Central Asia. Remarkably versatile, it has spread world-wide and has been cultivated for thousands of years as a source of food, fuel, fibre, medicine and as a narcotic [1]. Indeed, the link between humans and cannabis is so prevalent throughout recorded history that a study delving into the co-evolution of cannabis along with the human species has been advanced [2].

Due to millennia of cultivation and selective breeding, taxonomists have been unable to decide since the nineteenth century whether cannabis is best classified as a single species or as multiple species [3]. For instance, in 1753, Linnaeus considered cannabis as only one species; but during the year 2000, McPartland et al. described four species and in 2005, Hillig proposed seven putative taxa [4]. Probably unaware of this taxonomic disagreement, current recreational users classify cannabis into two types: sativa and indica.

Indica types are smaller in height, have broader leaves and typically mature faster under similar conditions than their sativa counterparts. Likewise, these two types of strains offer different highs. The indica high is described as a pleasurable body buzz with an overpowering sense of relaxation, calm and serenity. Users prefer this type to relieve overall body pain and for the treatment of insomnia. On the other hand, the sativa high is commonly characterized as energetic, uplifting and hallucinogenic. Users describe feelings of optimism and wellbeing [3]. Although both types of cannabis contain high contents of the principal psychoactive ingredient Δ-9 Tetrahydrocannabinol (THC), the sativa types are generally said to possess a higher concentration of

it. Conversely, cannabidiol (CBD), the second most important compound in the plant's essential oil, is more prevalent in the indica types [3].

Presently, marijuana is in a legal limbo of sorts in the United States. It is classified as a Schedule I substance under the Controlled Substance Act of 1970, which represents the highest tier in this legislation [5]. This means that the U.S. federal government has declared a high abuse potential and no established medical use for this herb. However, in 2012, Colorado and Washington became the first states to approve the recreational use of marijuana at the state level and other states have since followed suit. Currently, approximately two-thirds of the State Governments in the U.S. along with the District of Columbia have legalized marijuana use for either medical or recreational use [6].

This duality between federal and state legislations presents several logistical challenges for law enforcement, such as preventing the diversion of marijuana from states where it is legal to states where it is not and preventing legal marijuana operations from being used as a front for the trafficking of other drugs. Enforcement and interdiction are the chief methods utilized by law enforcement in this effort and in the gathering of intelligence of the current trafficking and movement trends. Therefore, a simple, fast and robust method to identify the source of seized marijuana samples would be beneficial to support law enforcement intelligence efforts and the forensic science community. Chemical profiling techniques using chromatographic techniques have been previously utilized in source determination and profiling of illicit drugs and may offer itself as a valuable tool in the geographical source-tracking of marijuana and its products.

The complex structure and chemistry of *C. sativa* L.

C. sativa L., the drug type cannabis this present study will focus on, consists of over 400 compounds representing a large variety of chemical classes. Among all these constituents and unique to this herb are C_{21} terpenophenolic compounds termed as 'cannabinoids' [6]. Selected structures of phytocannabinoids and terpenoids are shown in Figure 1.

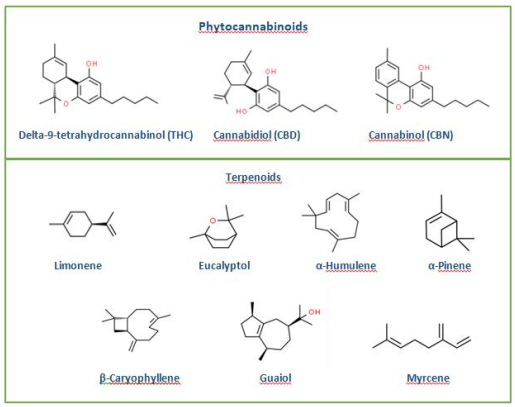

Figure 1: Structures of selected phytocannabinoids and terpenoids found in *C. sativa* L.

Due to the vast number of compounds and the numerous possible interactions between them, cannabis possesses a complex chemistry and is said to be a 'synergistic shotgun', as opposed to Marinol® (synthetic THC), termed as a single-ingredient 'silver bullet' [7]. Consequently, herbalists contend that the two advantages that cannabis has, as a polypharmaceutical herb, over single-ingredient synthetic drugs are: 1) the therapeutic effects of the primary active compounds may be synergized by other compounds, and 2) the undesirable side effects of the primary constituents may be mitigated by other compounds [7]. As such, four basic mechanisms of synergy have been proposed: 1) multi-target effects; 2) pharmacokinetic effects, such as improvised solubility or bioavailability; 3) agent interactions affecting bacterial resistance; and 4) modulation of adverse effects [8].

Phytocannabinoids

To make the distinction between the discovered endogenous cannabinoids and the development of synthetic cannabinoids, cannabis-based cannabinoids are termed as 'phytocannabinoids'. To date, there are 70 known phytocannabinoids. All are produced in secretory cells inside the glandular trichomes, which are most highly concentrated in unfertilized female flowers [9]. Due to their accumulation in these types of cells, phytocannabinoids serve chiefly as a defense system for the plant. It has been found that phytocannabinoids are cytotoxic compounds for cell suspension cultures from insects, suggesting insecticide properties [10]. Additionally, phytocannabinoids have also shown the ability to induce cell death through mitochondrial permeability transition in cannabis leaf cells, further suggesting a defense mechanism for cannabis leaves [11]. THC, CBD and CBN are phytocannabinoids, which play a major role in determining the plant's chemotype or chemical constitution.

THC is the best-known phytocannabinoid and the one most responsible for the plant's narcotic effects. It is usually present in a low concentration in raw and fresh cannabis; it is produced artificially from Tetrahydrocannabinolic Acid (THCA) during storage and by exposure to heat. Therefore, total THC content is the sum of the free THC naturally found in the plant material and the THC produced by the decarboxylation of THCA. This total THC content represents the maximum potency of the usually smoked, and thus decarboxylated, marijuana plant [12].

CBD is the next best studied and second most prevalent phytocannabinoid. Like THC, it is pharmacologically versatile, but less psychoactive than THC. Studies have shown that CBD positively modulates the adverse effects associated with THC, such as anxiety and tachycardia [6].

CBN is not naturally found in cannabis, it is actually the degradation product of THC and its presence indicates an aging plant. Assuming storage was carried out at room temperature, it is feasible to estimate the age of the plant based on its THC and CBN content [12].

Regardless of the breeding technique, phytocannabinoids are always present in a cannabis plant, albeit in differing concentrations. For instance, cannabis may be bred for agricultural and industrial purposes, or it may be bred for illicit purposes. The former is traditionally termed as 'fiber-type' cannabis and the latter as 'drug-type' cannabis. Fiber-type cannabis is characterized by a low THC content and a high CBD concentration; this relationship is reversed in drug-type cannabis. Drug-type cannabis is characterized by having THC content over 0.3% of inflorescence dry weight and a CBD level less than 0.5%. For cannabis to be classified as 'fiber' type and, thus be deemed legal, it has to have a THC content less than 0.2% in most European countries and 0.3% in Canada and in most states in the

United States [12]. Another chemotype, recognized as 'intermediate-type cannabis', has comparable levels of THC and CBD [13].

Studies have shown that the plant's chemotype never changes, despite its age, sex, origin or breeding method. THC in drug-type cannabis and CBD in fiber-type cannabis become the major cannabinoids as early as the first month of a plant's life, eliminating the need to wait for florescence in order to determine the chemotype of a seized sample [13,14].

Terpenoids

Among plant metabolites, terpenoids are the most abundant and comprise the largest group of chemicals found throughout the entire plant kingdom. They are commercially valuable as they have a wide range of applications in the cosmetic, food, pharmaceutical and fragrance industries [15]. This class of compounds is characterized by repeating isopropene units (C5H8) and can consist of simple linear chains or complex polycyclic structures, including functional groups, such as alcohols, ethers, aldehydes, ketones and esters. Terpenoids are also lipophilic, permeate lipid membranes, interact with neuronal and muscle ion channels, and many cross the blood-brain barrier [7].

Over 200 terpenoids have been reported in the essential oil of cannabis [16] and about 140 produce its typical musky scent [7]. Like phytocannabinoids, terpenoids are also produced inside the glandular trichomes and play a part in the plant's defense and communication mechanisms. For instance, monoterpenoids that act as repellants to herbivorous insects are found mostly in the flowers of cannabis while bitter sesquiterpenoids predominate in the leaves to deter grazing animals. Additionally, the content of mono- and sesquiterpenoids determines the viscosity of the essential oil of cannabis. The stickiness of this oil and the insecticidal phytocannabinoid acids found within provide the plant an excellent defense strategy against predators. Notably, although they represent 10% of the trichome content, terpenoid yield is less than 1% in most cannabis assays [16].

The diverse types of 'highs' and the different biochemical and pharmacological effects experienced by users consuming the numerous strains of marijuana are most likely related to the different terpenoid content and ratios [16,17]. Various mechanisms by which terpenoids regulate THC activity can be found in the scientific literature [18-20]. It should be noted that terpenoids with a concentration above 0.05% in the essential oil of cannabis are of pharmacological interest [16].

Review of the Literature: Methods of Source Tracking of Cannabis in Forensic Laboratories

As mentioned previously, interdiction is one of the main approaches used by law enforcement to track the trafficking of marijuana. Knowing the source of this illicit drug will prove beneficial to the law enforcement community as resources could then be assigned to where it is most needed.

Several attempts, including stable Isotope Ratio-Mass Spectrometry (IRMS), DNA profiling and chemical profiling, have been made in this endeavor with varying degrees of success, as shown in Figure 2. Marijuana is not chemically processed like other drugs, so it maintains its original elemental and isotopic profiles. This could be advantageous in indicating geographical origin since different growing conditions may affect the plant's composition of stable isotopes, such as 13C, 1H, 18O, 15N. Although it has been possible to source marijuana samples using Isotope Ratio-Mass Spectrometry (IRMS), this technique is still not common in forensic laboratories [21-23].

Method	Advantages	Limitations
Stable isotope ratio-mass spectrometry	• Different growing conditions results in different composition of stable isotopes	• Technique is not commonly available in forensic laboratories
DNA profiling	• Demonstrates genetic relationships (genotypes) • May be used to link, producers, traffickers and consumers	• Cloning of strains is commonplace, which interferes with source tracking efforts. • Expensive and labor intensive sample preparation • Limited access to street-seized samples • Comparative database has yet to be built
Chemical profiling	• Demonstrates chemotypes • Instrumentation commonly available in forensic laboratories • Reliable • Simple sample preparation • Same source = similar GC profiles • Non-destructive techniques available (HHS-SPME-GC/MS)	• Limited access to street-seized samples • Traditional GC/MS methods destructive • Natural variation and human intervention may potentially impart changes to the chemical profile

Figure 2: Comparison of profiling/geographic source tracking techniques.

DNA profiling has also been suggested as a potential tool in linking producers, traffickers and consumers based on the seized plant's genetic profile. However, the cloning of different strains and the selling of cuttings is quite common among growers. Thus, because a sample's DNA profile may not be unique, this technique is better apt for tracking cannabis samples derived from a common genetic lineage rather than for linking the evidentiary sample to a specific source. Moreover, this is a relatively expensive technique, and a comparative database of seized cannabis samples has yet to be constructed to estimate the expected frequency of a DNA profile match between unrelated plants [24, 25].

For chemical profiling, a Gas Chromatograph (GC) with a Mass Spectrometer (MS) or Flame Ionization Detector (FID) is used. Different cannabis specimens may demonstrate similar GC profiles, if they originate from the same source, but natural variation and human intervention may also potentially impart changes to the plant's GC profile. Nonetheless, correlation studies and likelihood ratios can link seized samples to a geographical origin if a reference material of known origin is available [12]. Although it is not always feasible to have authentic cannabis reference material, chemical profiling should be further explored, so it can be evaluated as a common and standardized analytical method for source determination of seized samples; especially since the instruments needed for these technique are already heavily used in forensic chemistry laboratories.

Chemical profiling of cannabis

Chemical profiling may offer information about the plant environment (i.e. growth conditions, chemicals present in the soil and use of regional pollutants or pesticides).Analogous to phytocannabinoids, terpenoid content also increases with exposure to light and decreases with soil fertility. Unlike its counterparts, which tend to reveal genetic relationships, terpenoids better reflect the plant's immediate environment [26], which is why several forensic studies

have been carried out to determine the feasibility of using terpenoid and phytocannabinoid chemical profiles to elucidate the geographic origin of confiscated marijuana. These studies, however, have provided mixed results.

Hood and Barry [27] reported a low relationship between headspace volatiles of cannabis and its origin. Brenneisen and ElSohly [28], conversely, determined that certain terpenes may be useful in the determination of the country of origin. Likewise, Hillig [17] reported differences in the terpenoid profile of cannabis from different origins, although some discrepancies between this study and Brenneisen and ElSohly's results were reported. Novak et al. [29] also reported distinct terpenoid differences in the essential oil composition of Eastern European and French cultivars of marijuana. Mediavilla and Steinemann [30] reported differences in terpenoid composition and aroma between different European hemp cultivars, but the data was not interpreted with respect to geographic origin. Similarly, Fischedick and his colleagues were able to chemically distinguish 11 different varieties of cannabis based on their cannabinoid and terpenoid contents [31]. However, in this particular study, data was not interpreted with respect to geographic origin.

Conclusion

Chemical profiling may lend itself as a valuable tool in assisting forensic interdiction efforts. However, future studies are needed to fully evaluate the efficacy and reliability of this technique in differentiating geographic sources of illicit marijuana evidence. Due to the complex nature of the chemical composition of marijuana, there is a need for further testing of marijuana plant samples of different known origins, which would allow for the creation of a chemometric database that could provide valuable geographic information for the intelligence community. Validation studies including samples with Inter-variation and intra-variation are also needed. Future research studies focused on comparing and contrasting the discriminatory power of chemical techniques to DNA techniques may contribute to the evolution of profiling techniques for illicit marijuana and its products. The coupling of chemical and biological methods may help to elucidate information about the sources of origin and environmental conditions for seized marijuana samples.

Additionally, with the decriminalization of cannabis and its products along with the widespread emergence of CBD products, there is an emerging necessity for clearly-defined quality control procedures to determine the chemical compositions of these samples quantitatively. Both, medical and recreational marijuana should be quality controlled. Cannabis products also need to be free of hazardous contaminants, such as mold and mites, consistent, and offer consumers the desired effect. There should also be infrastructure in place and regulations and laws enforced for testing and maintaining quality control in commercially sold marijuana. Strict guidelines should be in place from the cultivation of pot to its distribution to the consumers. Chemical profiling techniques may provide invaluable information to ensure those sources of recreational and medicinal marijuana and its products.

Limitations to Chemical Profiling for Marijuana Source Determination

Although chemical profiling may offer forensic laboratories the ability to utilize chemical signatures and fingerprints to determine the geographic source of marijuana, there are various limitations that must be addressed when evaluating the effectiveness of this potential tool. It has been previously reported that alterations in growth cycle and clipping of the lower branches of marijuana samples can cause quantitative differences in the chemical profile of cannabis plants grown under identical environmental conditions [32]. These differences can obscure the plant's chemical classification. Additionally, data sets derived from chemical profiling are complex and require multivariate analysis to correlate multiple variables simultaneously to determine possible trends or relationships between the target chemical marker compounds and source of origin. The lack of sensitivity and destruction of samples during analysis when using traditional gas chromatography-mass spectrometry profiling methods are also limitations. However, recently, the examination of chemical signatures of marijuana using non-destructive heated headspace solid phase micro extraction coupled with gas chromatography/mass spectrometry (HHS-SPME-GC/MS) and machine learning have been reported and may offer a suitable platform for advances in the field of chemical profiling [32].

Acknowledgements

The authors would like to thank the support of the Texas Southern University Forensic Science Learning Laboratory.

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