Review Article

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Approaches Helpful for Differentiating Hemp- and Marijuana-Derived Plant Products: A Review

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ABSTRACT: The legalization of hemp and the rapid growth of hemp-derived products have created major challenges for forensic and related laboratories in the United States. As marijuana remains illegal at the federal level and is heavily trafficked, accurately differentiating legal hemp from illicit marijuana is critical for law enforcement, industry, and medical applications. This review summarizes current methods used in cannabis classification, including morphological examination, chemical analysis, and genetic approaches, and discusses the limitations of each approach. Gas chromatography (GC)-based methods remain the primary technique in forensic laboratories due to their long-standing use in seized-drug analysis, efficiency, and cost-effectiveness. Liquid chromatography (LC)-based methods avoid thermal artifacts and allow the direct quantification of acidic and neutral cannabinoids. Other techniques, such as direct analysis in real time (DART) and spectroscopy, can also improve analytical efficiency. However, each approach has particular limitations, and the continuing emergence of hemp-derived cannabinoids interferes with analysis and interpretation. DNA-based approaches, for which synthase-gene markers, simple sequence repeat (SSR) markers, and organelle markers have been discovered and evaluated, can offer stable and environment-independent alternatives, yet their forensic implementation is limited by insufficient databases, assay complexity, and laboratory resource constraints. Continued development and method selection should be guided by laboratory resources and the intended application. It is important to understand the limitations and ensure careful evaluation and validation to support accurate and defensible cannabis testing.

KEYWORDS: Cannabis, chemical analysis, differentiation, genetic markers, hemp, marijuana, morphological analysis.

INTRODUCTION

Cannabis is one of the oldest cultivated plants, with evidence of its use for fiber, food, and medicine dating back thousands of years [103,169]. Although hard to pinpoint, its origins are often traced to Central Asia, whence it later spread to Europe by the first millennium BCE and to the Americas by the 16th century, becoming widely valued for its fibers, seeds, and medicinal properties [139].

Botanically, cannabis is an annual dioecious herb in the *Cannabaceae* family, closely related to *Humulus*. Within the *Cannabis* genus, *Cannabis sativa L.* (*C. sativa*) is commonly recognized as a singular species, while others such as *C. indica* (Lam.) and *C. ruderalis* (Janisch) are often treated as varieties, though debate remains over whether they represent distinct species or subspecific forms [68,99,138].

Given the ongoing taxonomic debate, many scholars favor classifying cannabis plants based on their biochemical profiles, particularly their cannabinoid composition [4,37,109,114]. Cannabinoids, specifically phytocannabinoids, are the most abundant class of compounds in cannabis. They are characterized by a C21 terpenophenolic backbone, with differences in side-chain length linked to the aromatic ring accounting for structural diversity among cannabinoid classes [120]. To date, more than 140 phytocannabinoids have been identified across 11 primary structural types: Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), cannabinol (CBN), cannabicyclol (CBL), cannabinodiol (CBND), cannabitriol (CBT), cannabielsoin (CBE), and miscellaneous-type cannabinoids. Among these, Δ^9-THC and CBD (**Figure 1**) are the most studied for their psychoactive and therapeutic properties [120,163]. Based on cannabinoid content, cannabis is commonly classified into five chemotypes:

- Drug-type plants with a predominant levels of Δ^9-THC [137];
- Plants with an intermediate ratio of Δ^9-THC to CBD (close to 1:1) [137];
- Fiber-type plants containing high levels of CBD and very low amounts of Δ^9-THC [137];
- Fiber-type plants that contain CBG as the main cannabinoid [52]; and
- Fiber-type plants that contain almost no cannabinoids [96].

Among these types, the most prominent distinction is between drug-type (marijuana) and fiber-type (hemp). Marijuana, rich in psychoactive Δ9-THC, is primarily used for medicinal and recreational purposes [6]; however, its narcotic effects have spurred controversy and ongoing debates over regulation for several decades. In

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Ya-Chih (Jessica) Cheng received her B.S. degree in food science and biotechnology from National Chung Hsing University (Taichung, Taiwan), an M.S. degree in toxicology from National Taiwan University (Taipei, Taiwan), and a Ph.D. degree in forensic science from Sam Houston State University (Huntsville, TX, USA). Dr. Cheng joined the Department of Forensic Science at The George Washington University (Washington, DC) as an assistant professor in August 2024. Dr. Cheng is also currently serving as a visiting scientist in the Organic Chemical Metrology Group and the Applied Genetics Group at the National Institute of Standards and Technology (NIST; Gaithersburg, MD).

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Dr. Cheng has authored/co-authored several peer-reviewed publications and presented her work at major conferences in forensic chemistry, forensic toxicology, and forensic genetics. Her contributions to the field have been recognized with multiple Emerging Forensic Scientist Awards from the Forensic Sciences Foundation, American Academy of Forensic Sciences. She is also an affiliate member of the American Academy of Forensic Sciences (AAFS).