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Cannabis cultivation: Methodological issues for obtaining medical-grade product

Suman Chandra [a], Hemant Lata [a], Mahmoud A. ElSohly [a,b], Larry A. Walker [a,c], David Potter [d,*]

[a] National Center for Natural Product Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, P.O. Box 1848, MS 38677, USA
[b] Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, P.O. Box 1848, MS 38677, USA
[c] Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, P.O. Box 1848, MS 38677, USA
[d] GW Pharmaceuticals plc, Sovereign House, Vision Park, Histon, Cambridge, CB24 9BZ, United Kingdom

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ABSTRACT

As studies continue to reveal favorable findings for the use of cannabidiol in the management of childhood epilepsy syndromes and other disorders, best practices for the large-scale production of *Cannabis* are needed for timely product development and research purposes. The processes of two institutions with extensive experience in producing large-scale cannabidiol chemotype *Cannabis* crops—GW Pharmaceuticals and the University of Mississippi—are described, including breeding, indoor and outdoor growing, harvesting, and extraction methods. Such practices have yielded desirable outcomes in *Cannabis* breeding and production: GW Pharmaceuticals has a collection of chemotypes dominant in any one of eight cannabinoids, two of which—cannabidiol and cannabidivarin—are supporting epilepsy clinical trial research, whereas in addition to a germplasm bank of high-THC, high-CBD, and intermediate type cannabis varieties, the team at University of Mississippi has established an *in vitro* propagation protocol for cannabis with no detectable variations in morphologic, physiologic, biochemical, and genetic profiles as compared to the mother plants. Improvements in phytocannabinoid yields and growing efficiency are expected as research continues at these institutions.

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1. Introduction

Plant-based drugs present unusual challenges in the pharmaceutical world with respect to large-scale cultivation, processing, quality, and consistency. In the case of *Cannabis sativa* L., considerable additional complexity derives from regulatory concerns, depending on the countries of production and marketing. In recent years, the production of cannabidiol (CBD)-based medicinal materials for research as potential therapeutics in childhood epilepsy syndromes and other disorders has come into greater focus. This article will describe the botany and pharmacognosy of *Cannabis sativa* L. and approaches employed in the United States and the United Kingdom (UK) for biomass selection, cultivation, and harvest/processing biomass to ensure quality supplies for medical research and for pharmaceutical product development.

2. Historical background in the United States and United Kingdom

In the United States, cannabis was common in patent medicines in the late 1800s and was listed in the US Pharmacopeia from the 1850s up until 1942, prescribed for various pain conditions and nausea. In 1899, cannabis was listed in the first edition of *Merck's Manual* [1] and recommended for the management of several conditions including epilepsy. It also had some history of use as an intoxicant, but, in 1937, the Marihuana Tax Act made it illegal except for medical use, which was taxed. Those who produced, prescribed, or dispensed marijuana were required to buy a stamp and pay the tax. This requirement greatly restricted legal use of marijuana, and, gradually, in the mid-20th century, when the use of most crude botanical drugs in US medicine declined, cannabis medical use plummeted as well. Of course, illicit use continued, but no "dealers" for this purpose would buy the stamp and pay the tax, because doing so would incriminate themselves. This practice was a major basis on which the Marihuana Tax Act was ruled unconstitutional in 1969. In 1970, congress passed legislation that included marijuana on the "Schedule I" list with many other controlled substances, and it remains so to this date.

* Corresponding author.
E-mail addresses: melsohly@olemiss.edu (M.A. ElSohly), djp@gwpharm.com (D. Potter).

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During the 1960s, the US government had also initiated a research program on marijuana, but the sources for the plant material were variable and unreliable. The National Institute of Mental Health contracted with the University of Mississippi (UM) School of Pharmacy in 1968 to grow marijuana for research purposes. This work has continued for more than 47 yr as a competitively awarded contract, popularly known for many years as "the nation's only legal marijuana farm." Today, UM supplies Good Manufacturing Practice (GMP) grade, high-quality marijuana plant material, along with extracts and purified cannabinoids, for the National Institute on Drug Abuse (NIDA) Drug Supply Program; this program makes the materials available to researchers studying their harmful and beneficial effects. In recent years, these activities have been specifically expanded to include CBD-enriched extracts of cannabis intended for clinical research use by qualified investigators. Many states (including Mississippi [2]) have passed legislation designed to allow clinical research and treatment of patients with epilepsy and other disorders, but the sourcing of these materials, from a federal regulatory perspective, has been a daunting challenge. The work reported in the present article was supported in part by NIDA, the National Institutes of Health, and the US Department of Health and Human Services, Contract No. N01DA-10-7793.

In the UK in the late 19th and early 20th century, the use of cannabis as a medicine saw a similar rise and fall to that seen in the United States. Its use declined, however, and ceased when declared a Schedule I substance in 1971. In 1998–99, two major official investigations were carried out into cannabinoid science and, more broadly, the issues related to the potential medical benefits of cannabis. The investigations were performed by the House of Lords Science and Technology Select Committee in the UK and the Institute of Medicine's National Academy of Sciences in the United States [3–5]. Both of these investigations found strong evidence supporting the potential therapeutic effects of components of the *Cannabis* plant, particularly in the field of multiple sclerosis and pain management. GW Pharmaceuticals, formed in the UK in 1998, immediately set about addressing these issues. From its inception in 1998, however, GW also had a desire to develop plants for the possible future treatment of patients with epilepsy [6]. For the following 12 yr, however, all of GW's commercial crops were grown for production of Sativex®, a botanical drug used for managing symptoms of multiple sclerosis. More recently, the company has been developing the drug Epidiolex®, a liquid formulation of pure plant-derived CBD as a treatment for patients with various orphan pediatric epilepsy syndromes. The US Food and Drug Administration (FDA) has granted Epidiolex® orphan status for the treatment of patients with Dravet syndrome, Lennox-Gastaut syndrome, infantile spasms (West syndrome), and tuberous sclerosis complex, as well as a fast-track designation for Dravet syndrome.

The company is also performing clinical trials with a drug that features cannabidivarin (CBDV) as the primary cannabinoid. This drug has shown antiepileptic properties across a range of preclinical models of epilepsy. GW has advanced research efforts of the experimental CBDV-based drug GWP42006 into a Phase 2 study of epilepsy.

3. Cannabis physiology

Cannabis is predominantly an annual herb of central Asian origin, which, heavily influenced by man over several millennia, has adapted to grow in almost all parts of the world, from the tropics to the edge of the Arctic Circle [7]. It is one of the oldest plant sources for food, textile fiber, and medicine. It is only within the last century that the species has also become synonymous with use as a recreational drug.

In addition to its direct use as a foodstuff, cannabis seeds can be crushed to produce oil for a variety of purposes. This oil can contain large quantities of essential fatty acids, especially *gamma*-linolenic acid and stearidonic acid. Because of the presence of these highly valuable nutrients, hemp seed oil is widely marketed as a health food supplement. Hemp seed oil is not to be confused with the pharmaceutical material CBD oil, as dispensed at UM. CBD oil is made by solvent extraction of CBD-rich female cannabis flowers, which is then dissolved in an oil. It should also not be confused with hash oil, a liquid or semisolid concentrated extract of *Cannabis* plant material [8]. This is used recreationally, but those in possession of illicit samples often claim to have them for medicinal purposes.

Cannabis is predominantly dioecious, with male and female flowers by definition developing on separate plants if grown naturally from seed. It does, however, occasionally exhibit a monoecious (hermaphrodite) nature. Varieties bred specifically for fiber production are predominantly hermaphrodite, as this characteristic produces more uniform material.

Apart from plants derived from extreme equatorial or polar provenance, *Cannabis* is normally a so-called "short-day plant." Such plants naturally commence flowering at the end of summer in response to a detected increase in night length. In the past 15 yr, increasing numbers of so-called autoflowering varieties have become commercially available. These plants are not day-length sensitive and commence flowering when only approximately 2 wk old, irrespective of the day length. They can be grown in continuous lighting, producing very high yields. GW's research program includes trials with CBD chemotype plants of this type.

In general, it is impossible to discriminate male plants from female plants at the vegetative stage. However, flowering male and female plants can be easily differentiated based on their very different floral structures. Although of limited application, molecular techniques allow male and female plants to be differentiated at an early growth stage [9–11].

Cannabis sativa L. is a wind-pollinated species, which is highly allogamous in nature. A significant amount of plant-to-plant variation in its cannabinoids profile and content is observed, even when the crop is propagated through a single seed accession. For the production of cannabinoids, all-female crops are preferred. Male plants produce much lower quantities of cannabinoids, and pollinated females divert resources away from cannabinoid production for seed development. To avoid this process, one option is to remove male plants as they appear. Alternatively, the presence of male plants can be prevented by using vegetative propagation and/or micropropagation while ensuring in each case that the propagation material is female. It is also possible to propagate crops from specifically produced all-female seeds. The latter two practices are described in Section 6.

4. Cannabinoid biosynthesis

Cannabis sativa L. is considered a chemically complex species based on its numerous natural constituents. It contains a unique class of terpenophenolic compounds (cannabinoids or phytocannabinoids) that have been extensively studied since the discovery of the chemical structure of tetrahydrocannabinol (Δ^9-THC), commonly known as THC, the main constituent responsible for the psychoactive effects of cannabis. A total of 565 constituents, including 120 phytocannabinoids, have been reported in *Cannabis* so far [12]. Besides Δ^9-THC, CBD, and CBDV, other major cannabinoids of *Cannabis*, including Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) are showing potential pharmaceutical interest. In fresh plant material, these cannabinoids all exist in the cannabinoid acid forms, such as Δ^9–tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA). As the plant material ages or is heated, the acid molecules lose a carboxyl moiety. Decarboxylation results in the conversion of the cannabinoid acids into their neutral forms (e.g., CBDA → CBD). As is common, this article hereafter refers to the cannabinoids in their neutral form only.

The cannabinoids are predominantly, if not entirely, synthesized and sequestered in small structures called glandular trichomes [13]. Evidence suggests that these secondary metabolites play a major role in the defense of the cannabis plant. Plant species generally tend to optimize defense by allocating secondary metabolites