Cannabidiol (CBD) From Non-Cannabis Plants: Myth or Reality?

Giovanni Appendino¹, Orazio Taglialatela-Scafati², and Eduardo Muñoz³

Abstract
Reports on the occurrence of cannabidiol (CBD, 1) in non-cannabis plants are critically reviewed. The isolation of 1 from Humulus Kriya (sic) was fraudulent and from Trema orientalis and stevia dubious, while the occurrence of traces of 1 in flax needs additional confirmation. The presence of high concentration of cannabigerol (CBG, 3a) and its corresponding acidic precursor (GBGA, 3b) in Helichrysum umbraculigerum could not be confirmed, but this plant deserves additional attention due to the possible phytocannabinoids accumulation in selected chemotypes.

Keywords
cannabidiol, cannabinoids, cannabis, flax, stevia, Helichrysum umbraculigerum, Trema orientalis, Humulus kriya

Received: March 14th, 2022; Revised: April 14th, 2022; Accepted: April 19th, 2022.

The FDA and EMA approval of cannabidiol (CBD, 1) for the management of 2 rare genetic forms of epilepsy (Lennox-Gastaut and Dravet syndromes)¹ has triggered a substantial shift of interest in the medical community from the narcotic phytocannabinoid Δ⁹-THC (Δ⁹-THC, 2) to its non-narcotic structural analogue cannabidiol (CBD, 1).²

According to FDA, natural CBD from hemp is an Active Pharmaceutical Ingredient (API), and therefore not a dietary ingredient.³–⁵ Nevertheless, despite its unclear regulatory status, CBD has become a household name in the US dietary supplement market, to the point that a 2019 Gallup poll disclosed that one in seven American adults was using a CBD-based product.⁶ The anti-epileptic target(s) of CBD are still unclear,¹,² but the affinity of CBD for a multitude of end points,⁷ although generally modest, has nevertheless made it easy to support its potential beneficial activity in a wide range of therapeutic areas, including the COVID-19 pandemic.⁸

The obtaining of CBD by isolation from hemp (Cannabis sativa L.) is complicated by the legal constraints associated with the co-occurrence of Δ⁹-THC, an issue that conventional breeding has faced for decades, and that only the development of engineered transgenic plants seems to have eventually solved.⁹ Synthesis can provide CBD at a lower price than isolation from Cannabis, but synthetic CBD is a Schedule 1 compound in the United States (but not in the EU).³–⁵ Because of legal issues surrounding synthetic CBD and Cannabis in general, the identification of an alternative botanical source of this compound could be useful to dissect CBD, a non-narcotic compound, from its potentially narcotic plant source. We discuss here the possibility that CBD could be isolated from a non-Cannabis plant, critically analyzing the claims that this has been achieved.

The biosynthetic logic used by C sativa to produce cannabinoids is not unique to this plant, whose singularity is exclusively related to the nature of the building blocks used. Thus,
cannabinoids originate from the decoration of a polyketide-derived substituted resorcinolic core with an isoprenoid group (Scheme 1). Most cannabinoid-producing plants use an aryl-containing starting unit for building the resorcinolic core by iterative addition of acetate units, followed by aldol-type aromatization, resulting in the formation of a phenethyl-substituted resorcinol moiety. Conversely, *C. sativa* exclusively uses alkyl starters for the iterative growth of the ketide chain, resulting, after aromatization, in cannabinoids with a linear alkyl substituent (Scheme 1). The latter is generally a pentyl group (cannabinolo-olivetoids) with shorter chains [cannabinobutoids (C-4)], cannabinoids with isoprenoid decoration of the aromatic core is not derived from a geranyl precursor, but from a farnesyl (C-15) unit, a biogenetic pattern so far reported in a single Cannabis phytocannabinoid (sesquicannabigerol). In conclusion, Cannabis phytocannabinoids could, in principle, also occur in other plants, but the amount detected was extremely low compared to the ones occurring in Cannabis, in the range of 10 ppm in seeds, the richest organ. Given the similarity of the MS fragmentation pattern within phytocannabinoids, the identification could have greatly benefited from the actual isolation of the CBD. Furthermore, activity in the bioassays investigated (pattern of inflammatory gene expression in human and murine fibroblasts, skin fibroblasts, and keratinocytes proliferation potential) was not specific for cannabinoids. Since only trace amounts of CBD were detected, the possibility of soil-mediated horizontal natural product transfer could not be ruled out. Natural products can leach from plant tissues in the soil, and the absence of a background contamination from previously grown hemp needs to be taken into due consideration. Given the anti-bacterial and anti-inflammatory activity of cannabinoids, their presence in flax fiber has relevance for its use in wound-dressing, but additional proof would be necessary to back up these preliminary results, namely the actual isolation of the major flax cannabinoid, its unambiguous identification with CBD, and the detection of active genes for cannabinoid biosynthesis in flax.

CBD was reported to co-occur in *H. umbraigerum* with minor amounts of phenethyl-type analogues, all characterized by a linear terpenyl residue. On the other hand, a more recent investigation of *H. umbraigerum* from the Johannesburg botanical garden only afforded phytocannabinoids of the phenethyl type. The detection of a different phytochemical profile could be related to the different origin of the plant material, and *H. umbraigerum* deserves, therefore, additional investigation, being the first, and so far the only plant outside of *C. sativa*, where the isolation, and not simply the detection, of Cannabis phytocannabinoids has been unambiguously reported.

In 2012, CBD was detected in trace amounts in the hydrophobic fraction of *Linum usitatissimum* L. fiber, seeds, and whole aerial parts. CBD was detected both in wild plants and, in slightly higher concentration, also in a transgenic accession (W92) where 3 genes from the flavonoid biosynthetic cluster had been overexpressed to increase the production of anti-oxidants and improve pest resistance. CBD was identified by mass spectrometry, co-injection with a standard in UPLC and GC profiles, as well as by bioassay. However, the amounts detected were extremely low compared to the ones occurring in Cannabis, in the range of 10 ppm in seeds, the richest organ. Given the similarity of the MS fragmentation pattern within phytocannabinoids, the identification could have greatly benefited from the actual isolation of the CBD. Furthermore, activity in the bioassays investigated (pattern of inflammatory gene expression in human and murine fibroblasts, skin fibroblasts, and keratinocytes proliferation potential) was not specific for cannabinoids. Since only trace amounts of CBD were detected, the possibility of soil-mediated horizontal natural product transfer could not be ruled out. Natural products can leach from plant tissues in the soil, a well-known problem for nicotine contamination from cigarette butts, and the absence of a background contamination from previously grown hemp needs to be taken into due consideration. Given the anti-bacterial and anti-inflammatory activity of cannabinoids, their presence in flax fiber has relevance for its use in wound-dressing, but additional proof would be necessary to back up these preliminary results, namely the actual isolation of the major flax cannabinoid, its unambiguous identification with CBD, and the detection of active genes for cannabinoid biosynthesis in flax.

In 2012, CBD was detected in trace amounts in the hydrophobic fraction of *Linum usitatissimum* L. fiber, seeds, and whole aerial parts. CBD was detected both in wild plants and, in slightly higher concentration, also in a transgenic accession (W92) where 3 genes from the flavonoid biosynthetic cluster had been overexpressed to increase the production of anti-oxidants and improve pest resistance. CBD was identified by mass spectrometry, co-injection with a standard in UPLC and GC profiles, as well as by bioassay. However, the amounts detected were extremely low compared to the ones occurring in Cannabis, in the range of 10 ppm in seeds, the richest organ. Given the similarity of the MS fragmentation pattern within phytocannabinoids, the identification could have greatly benefited from the actual isolation of the CBD. Furthermore, activity in the bioassays investigated (pattern of inflammatory gene expression in human and murine fibroblasts, skin fibroblasts, and keratinocytes proliferation potential) was not specific for cannabinoids. Since only trace amounts of CBD were detected, the possibility of soil-mediated horizontal natural product transfer could not be ruled out. Natural products can leach from plant tissues in the soil, a well-known problem for nicotine contamination from cigarette butts, and the absence of a background contamination from previously grown hemp needs to be taken into due consideration. Given the anti-bacterial and anti-inflammatory activity of cannabinoids, their presence in flax fiber has relevance for its use in wound-dressing, but additional proof would be necessary to back up these preliminary results, namely the actual isolation of the major flax cannabinoid, its unambiguous identification with CBD, and the detection of active genes for cannabinoid biosynthesis in flax.

CBD was reported to occur, along with Δ9-THC (2) and cannabinol (CBN), in the inflorescences of *Tremna orientalis* L., a shrub distributed in tropical Africa and Asia and used in folk medicine as an anti-infective agent. *Tremna* is one of the 8 genera that recent taxonomic work has moved from the Celtidaceae family, which now contains about 170 species. Three collections of the plant from different locations in Thailand were investigated, and not all contained CBD. Cannabinoid identification was made by comparison of retention time in HPLC and GC analysis. The content of CBD was very low (ca 5 ppm), but the one of THC and CBN was higher. Nevertheless, the authors did not isolate any pure phytocannabinoid, something that would have surely strengthened their findings. In addition, the article over-emphasizes the taxonomic relationship between the genus *Tremna* and the genus *Cannabis*. Also worth mentioning is that previous investigations of other
plant parts of *T. orientalis* did not disclose the presence of compounds biogenetically related to phytocannabinoids.\(^{24,25}\)

The claim that CBD occurs in high concentration in *Humulus Kriya* (sic) is a remarkable example of forgery and fraud, worth recapitulating to give an idea on how commercial interests and a largely unregulated market like the one of “dietary” phytocannabinoids can foster pseudoscience. In 2018, a claim was made on the generation, by conventional painstaking hybridization, of a hops chemovar containing high concentration of cannabinoids. Wild samples of *Humulus yunnanensis* Hu, one of the three known hops species, were collected in different Himalayan locations of India, and screened for the presence of phytocannabinoids.\(^{26}\) The hit rate was allegedly very poor (one hit every 800-1000 samples analyzed), and the contents very low. Nevertheless, some accessions with unusually high contents were discovered, fostering breeding work that eventually generated the chemovar *H. Kriya*.\(^{26}\) The phytocannabinoid profile of this plant was characterized by a high concentration of CBD (1), while the contents of \(\Delta^9\text{-THC} (2)\) were below the analytical detection threshold.\(^{26,27}\) The CBD-expressing gene was apparently recessive, and this research was published in the first issue of the *Journal of Medicinal Phyto Research*, an ad hoc created journal.\(^{26}\) A vis-à-vis comparison of extracts from different plant parts of various strains of *C. sativa* and *H. Kriya* showed comparable activity, and, presumably, concentrations as well. The presence of CBD in *H. Kriya* was supported by its isolation and crystallographic analysis, and not simply by its analytical detection.\(^{27}\) Bioactivity of CBD from *H. Kriya* was evaluated using a monoclonal antibody test (sic), as well as displacement assays from CB2 with radiolabeled ligands.\(^{26}\) The authors of the two articles also bemoaned that comparison with *CBD from yeasts* (sic) as well as displacement assays from CB2 with radiolabeled ligands.\(^{26}\) The authors of the two articles also bemoaned that comparison with “transgenic” CBD from yeasts was not possible because of the unavailability of “transgenic” CBD. The development of *H. Kriya* was also protected by a patent.\(^{26}\) *H. Kriya* was claimed to be the first legal non-narcotic source of phytocannabinoids on the reasoning that it does not contain \(\Delta^9\text{-THC} (2)\), and that hops belong to a family of plants considered GRAS, with *H. Kriya* having also been allegedly certificated as a Food Ingredient by the Food Safety and Standards Authority of India.\(^{26} \) In other words, *H. Kriya* was, for the first time, removing from CBD the stigma associated with its origin from a narcotic plant species, allegedly solving the legal ambiguity surrounding this compound.

From the very beginning, the whole issue of *H. Kriya* seemed untenable from a regulatory standpoint and highly dubious in scientific terms, with nothing supporting the rhetoric used by two companies to advertise their hops-derived CBD products.\(^{29}\) Thus, Federal Government restrictions in the United States regard CBD itself, independently of its derivation,\(^{33-35}\) and no loophole therefore exists to bypass the regulation using a non-Cannabis source of this compound. Additionally, since hops lack the biochemical machinery to produce phytocannabinoids, genetic modification seems the only way to get hops containing these compounds.\(^{30}\) Hops and hemp share a series of characters: both are naturally diploid, dioecious, wind-pollinated, and present glandular trichomes on their inﬂorescences. On the other hand, except for the co-occurrence of some volatile terpenoids like humulene and \(\beta\)-caryophyllene, their phytochemistry is very different.\(^{23-26}\) Both these Cannabaceae plants accumulate terpenophenolic, but hops use branched amino acids as starters for the formation of a phloroglucinol core via Claisen-type aromatization, while hemp uses acetate-derived building blocks for the formation of a resorcinolic core via an aldol-type aromatization reaction.\(^{40}\) Next, isoprenylation of hop phloroglucinols uses an isoprenyl group, while the same decoration in hemp resorcinols uses a geranyl moiety. Eventually, a careful analysis of the two articles showed that they had been both fabricated by plagiarizing near word-by-word published literature on CBD, confirming the skepticism of the scientific community.\(^{29}\) Also, the researcher who claimed to have discovered *H. Kriya* turned out to be a convicted con artist well-known to the police.\(^{29}\)

In the proprietary literature, a patent claims the isolation of CBD from the floral leaves of stevia (*Stevia rebaudiana* Bertoni), an asteraceous plant taxonomically unrelated to *H. umbra- calyx*. The stevia variety SrUGT771E1 had previously been found capable of glucosylating olivetolic acid, cannabigerolic acid, and \(\Delta^9\text{-tetrahydrocannabinolic acid, but not to produce phytocannabinoids.}^{32}\) suggesting the need of additional studies on the occurrence of CBD in stevia.

In conclusion, despite the non-unicity of the biosynthetic logic underlying the production of phytocannabinoids by *C. sativa*, the nature of the building blocks used for the assembly of the resorcinolic core and for its decoration with isoprenoid residues is responsible for the paucity of reports on the “ectopic” occurrence of Cannabis phytocannabinoids in Nature, with *H. umbra-calyx* remaining the only other plant from which compounds of this type have been isolated. The detection of CBD in flax, in *T. orientalis*, and in stevia needs additional confirmation studies, while the whole issue of *H. Kriya* is simply a story of greed and scientific forgery. A final issue worth attention is the absolute configuration of a hypothetical non-Cannabis CBD. Thus, CBD from Cannabis has a very high enantiomeric purity with a 3R,4R configuration (p-methane numbering),\(^{33}\) but *cis*-\(\Delta^9\text{-THC} (5)\) from Cannabis and its phenethyl analogue per- oxetene (6) from various *Rastula* species\(^{34-36}\) have an opposite configuration at C-6a (corresponding to C-4 in the p-methane numbering system). This suggests that, in the search for non-Cannabis sources of CBD, the relative configuration of the 2 stereogenic centers, as well as the absolute configuration and the optical purity need to be critically evaluated.
space) for financial support. The authors would like to dedicate this manuscript to Prof. Yoshinori Asakawa for his outstanding achievement in natural products research.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Ministero dell’Università e della Ricerca (grant number PRIN2017, Project 2017WN73PL).

Ethical Approval
Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

References
1. Golub V, Reddy DS. Cannabidiol therapy for refractory epilepsy and seizure disorders. Adv Exp Med Biol. 2021;1264:93-110.
2. Cristiano I, Bisogno T, Di Marzo V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. Nat Rev Neurol. 2020;16(1):9-29.
3. Citti C, Linciano P, Cannazza G. Is cannabidiol a scheduled controlled substance? Origin makes the difference. Drug Discov Today. 2020;25(4):628-632.
4. Vlad RA, Hancu G, Ciurba A, et al. Cannabidiol-therapeutic and legal aspects. Pharmazie. 2020;75(10):463-469.
5. Brunetti P, Lo Faro AF, Pirani F, et al. Pharmacology and legal status of cannabidiol. Ann Ist Sup San. 2020;56(3):285-291.
6. Gallup 2019; June 19-July 12 https://news.gallup.com/poll/263147/americans-say-cbd-products.aspx.
7. Nelson KM, Bisson J, Singh G, et al. The essential medicinal chemistry of cannabidiol (CBD). J Med Chem. 2020;63(21):12137-12155.
8. Malinowska B, Baranowska-Kuczko M, Kieman A, et al. Opportunities, challenges and pitfalls of using cannabidiol as an adjuvant drug in COVID-19. Int J Mol Sci. 2021;17(4):1986.
9. The Colorado company, Trilogene Seeds, reported in January 2022 the successful development of Pandora, a C. sativa strain where, using RNA interference, the production of Δ9-THC has been shut down (https://www.chemspro.com/copy-of-logical-greensolutions).
10. Hanuš LO, Meyer SM, Muñoz E, et al. Phytocannabinoids: a unified critical inventory. Nat Prod Rep. 2016;33(12):1349-1351.
11. Linciano P, Citti C, Luongo I, et al. Isolation of a high-affinity cannabinoid for the human CB1 receptor from a medicinal cannabis sativa variety: Δ9-Tetrahydrocannabivarin, the butyl homologue of Δ9-tetrahydrocannabinol. J Nat Prod. 2020;83(1):88-98.
12. Citti C, Linciano P, Russo F, et al. A novel phytocannabinoid isolated from Cannabis sativa L. with an in vivo cannabinimimetic activity higher than Δ9-tetrahydrocannabinol: Δ9-Tetrahydrocannabiphorol. Sci Rep. 2019;9(1):20335.
13. Pollastro F, Caprioglio D, Del Prete D, et al. Cannabichromene. Nat Prod Commun. 2018;13(9):1189-1194.
14. Pollastro F, Tagliatela-Scalfati O, Allara M, et al. Bioactive prenyllogous cannabinoid from fiber hemp (Cannabis sativa). J Nat Prod. 2011;74(9):2019-2022.
15. Bohlmann F, Hoffmann E. Cannabigerol-ähnliche Verbindungen aus Helichrysum umbracluigerum. Phytochemistry. 1979;18(8):1371-1374.
16. Lourens ACU, Viljoen AM, Van Heerden FR. South African Helichrysum species: a review of the traditional uses, biological activity and phytochemistry. J Ethnopharmacol. 2008;119(3):630-652.
17. Botha KS, Crampton BC, Heyman HM. The in vitro propagation of Helichrysum umbracluigerum. Planta Med. 2015;81(3):236.
18. Pollastro F, De Petrocellis L, Schiano-Moriello A, et al. Amorfrutin-type phytocannabinoids from Helichrysum umbracluigerum. Fitoterapia. 2017;123(1):13-17.
19. Styrczewska M, Kulma A, Ratajczak K, et al. Cannabinoid-like anti-inflammatory compounds from flax fiber. Cell Mol Biol Lett. 2012;17(3):479-499.
20. Selmar D, Radwan A, Abdalla N, et al. Uptake of nicotine from discarded cigarette butts – A so far unconsidered path of contamination of plant-derived commodities. Environ Pollut. 2018;238(1):972-976.
21. Styrczewska M, Kostyn A, Kulma A, et al. Flax fiber hydrophobic extract inhibits human skin cells inflammation and causes remodeling of extracellular matrix and wound closure activation. BioMed Res Int. 2015: Article ID 862391.
22. Naparro T, Tanruca K, Poolsraset P, et al. Cannabidiol from inflorescences fractions of Trema orientalis (L.) Blume (Cannabaceae) against human pathogenic bacteria. Peer J. 2021;1–6.
23. McPartland JM. Cannabis systematics at the level of family, genus, and species. Cannabis Cannabinoids Res. 2018;3(1):203-212.
24. Dijoux-Franca MG, Nougué TD, et al. New dihydrophenanthrene and phenylhydroisocoumarin constituents of Trema orientalis. J Nat Prod. 2001;64(6):832-835.
25. Kuo W-I, Huang Y-L, Wang S-T, et al. Chemical constituents of Trema orientalis. J Chin Med Sci. 2007;2(1):27–36.
26. Cushing D, Kristipati S, Shastri R, et al. Measuring the bioactivity of phytocannabinoid cannabidiol from cannabis sources, and a novel non-cannabis source. J Med Phyto Res. 2018;1(1):10-25.
27. Cushing D, Joseph B. Identification of cannabidiol from Humulus Krija using x-ray crystallography. J Med Phyto Res. 2018;1(1):29-47.
28. USPP31,477P3. The patent claims a phytocannabinoid contents of 12–14% in the inflorescences of H. Kryia, as well as a contents of ca 1% of β-caryophyllene in the leaves.
29. https://www.poiternetwork.com/ (Researcher Who Claimed CBD In Hops Revealed As Convicted Con Artist. February 26, 2019)
30. Arif Y, Singh P, Baiguz A, Hayat S. Phytocannabinoids biosynthesis in angiosperms, fungi, and liverworts and their versatile role. *Plants*. 2021;10(7):1307.

31. Liu S, Wang Z, Hongfen X et al. Highly efficient purification of cannabidiol from floral leaf of *Stevia rebaudiana* Bertoni. 2021, CN113754518 A 2021-12-07.

32. Gülck T, Booth JK, Carvalho À, et al. Synthetic biology of cannabionoids and cannabinoid glucosides in *Nicotiana benthamiana* and *Saccharomyces cerevisiae*. *J Nat Prod*. 2020;83(10):2877-2893.

33. Mazzoccanti G, Ismail OH, D’Acquarica I, et al. Cannabis through the looking glass: chemo- and enantio-selective separation of phytocannabinoids by enantioselective ultra high performance supercritical fluid chromatography. *Chem Commun*. 2017;53(91):12262-12265.

34. Schafroth MA, Mazzoccanti G, Reynoso-Moreno I, et al. Δ⁹-Δ⁹-Tetrahydrocannabinol: natural occurrence, chirality, and pharmacology. *J Nat Prod*. 2021;84(9):2502-2510.

35. Chieca A, Schafroth MA, Reynoso-Moreno I, et al. Uncovering the psychoactivity of a cannabinoid from liverworts associated with a legal high. *Sci Adv*. 2018;4:eaaat2166.

36. Toyota M, Shimamura T, Ishii H, et al. New bibenzyl cannabinoids from the New Zealand liverwort Radula marginata. *Chem Pharm Bull*. 2002;50(10):1390-1392.