Pharmacy Education (2024) 24(6) 43 - 49
https://doi.org/10.46542/pe.2024.246.4349

ICOPMAP SPECIAL EDITION

RESEARCH ARTICLE

Identification of Δ9-tetrahydrocannabinol compounds in Cannabis sativa using gas chromatography-mass spectrometry

Gilang Bhakti Nusantara, Tesia Aisyah Rahmania
Faculty of Military Pharmacy, Republic of Indonesia Defense University, Bogor, Indonesia

Keywords
Cannabis sativa
Duquenois
GC-MS
Narcotic
Narcotic abuse

Correspondence
Tesia Aisyah Rahmania
Faculty of Military Pharmacy
Bogor Regency
West Java
Indonesia
tesiaaisyah4@gmail.com

Abstract

Background: Narcotics are divided into three types natural, semisynthetic and synthetic. Cannabis is included in the Cannabaceae family with the species Cannabis sativa L. This plant is known as a plant that has psychotropic effects because it contains the main alkaloids cannabinol, cannabidiol, and Δ9-Tetrahydrocannabinol. **Objective:** This study aims to identify the presence of Δ9-Tetrahydrocannabinol in samples. **Method:** The method used is the Duquenois colour test, Fast Blue, Gas Chromatography-Mass Spectrometry (GC-MS) confirmation. **Result:** The result shows that Dequenoise test positive giving a blue color and Fast Blue color test positive giving a brick red. The GC-MS analysis shows the presence of Δ9-Tetrahidrocannabivarin, Cannabichrome, Dronabinol, Cannabigerol, and Cannabinol. The chromatogram revealed five peaks, with the main compound identified as dronabinol. **Conclusion:** Five compounds found in sample were 9-tetrahydrocannabivarin, cannabichromene, dronabinol, cannabigerol, and cannabinol, which were positive for marijuana.

Introduction

Based on the latest report, the National Narcotics Agency of the Republic of Indonesia (BNN RI) has confiscated 78.4 Kg of methamphetamine and 62 Kg of marijuana from 15 cases of narcotics crime spanning May to July 2022 (Puslitdatin BNN, 2022). According to the United Nations Office On Drugs and Crime (UNODC) World Drug Report 2022, the global number of marijuana users aged 15-64 years reached around 209 million in 2020, indicating a significant 4% increase from 2019 (United Nations Office on Drug and Crime, 2022). In line with these figures, the 2021 National Survey on Drug Abuse in Indonesia highlighted that marijuana-type drugs are in the top position, with 41.1% of users (Puslitdatin BNN, 2022). Based on the surge in marijuana confiscation cases in Indonesia, there is a need for a robust testing method to identify marijuana. This report will discuss cannabis testing using colour and confirmatory tests using gas chromatography-mass spectrometry (GC-MS), applied in routine testing at the National Narcotics Laboratory Center of the National Narcotics Agency.

According to Law No. 35 of 2009, narcotics are substances or drugs derived from plants or non-plants, including synthetic and semi-synthetic compounds that can cause a decrease or change in consciousness, taste loss, pain reduction or elimination, and dependence. The term narcotics derives from the word narcosis, which means anaesthesia; hence, these agents also include anaesthetics (Isnaini, 2017). The World Health Organisation (WHO) defines drugs as any substance — apart from food, water, and oxygen— that, when ingested, alters the body's physiological and/or psychological processes. Narcotics can be of natural, synthetic, or semi-synthetic origin.

Narcotics of natural origin are directly obtained from nature. Examples include cannabis derived from the Cannabis sativa plant, opium obtained from Papaver somniferum, and psilocybin extracted from cow dung mushrooms. Semi-synthetic narcotics are obtained from natural materials and then processed with

chemicals to form processed drugs. For example, opium is synthesised into heroin, morphine, or codeine by adding chemicals, while coca leaves produce cocaine powder after adding potassium permanganate. The third category, i.e. synthetic narcotics, includes all types of drugs produced through the synthesis of drug substances or other chemicals. An example is the amphetamine-type stimulant (ATS) class, with methamphetamine synthesised using ephedrine/ pseudoephedrine P2P precursors and MDMA produced with safrole/isosafrole/MDP2 precursors.

In addition to being categorised into natural, synthetic, or semi-synthetic, narcotics are also divided into three classes (Undang Undang RI, 2009).
Class I narcotics are exclusively used in research and are not intended for therapeutic uses. They have an exceptionally high potential to cause dependence. Class II narcotics can be used for science development and are the last resort for therapy.
Class III narcotics can be employed in therapy and scientific research. They carry a mild potential for inducing dependence.

Cannabis, known as Cannabis sp and belonging to the Cannabaceae family (McPartland, 2018), is classified under the Cannabis genus with Cannabis sativa L (Thomas & ElSohly, 2016). Morphologically, this plant consists of stems, leaves, flowers, and fruits or seeds (Thomas & ElSohly, 2016). Cannabis stems have a green colour, a hollow upright structure, a cylindrical shape, and longitudinal grooves, reaching heights of 1-3 meters with lower branches extending to 1.2 meters. The shoots are long and thin (Chandra *et al.,* 2017).

Cannabis leaves are finger-shaped, consisting of 3-9 strands (always odd) with a linear lanceolate leaf shape measuring 3-15 x 0.2-1.7cm. The leaf edges are serrated towards the tip; the upper leaf surface is rough (scabrid) with feathers at the base, while the lower leaf surface is strigose (abaxial) pale green and covered with yellow resin dots. The leaf arrangement changes from opposite (decussate) to switch up at the plant's tip, and the petioles are 2-7cm long with a narrow groove (United Nations Office on Drug and Crime, 2022a).

Cannabis flowers consist of male and female varieties. Male flowers are pale green and have five white-green downy sepals 2.5-4mm long and five dangling stamens. Female flowers are dark green, tightly gathered at the top, and include a pollination site with a long, slender, hairy stigma on each flower (Thomas & ElSohly, 2016).

Cannabis fruits or seeds, measuring 4-6mm long and 3-4mm in diameter, are smooth, slightly dense, brownish-grey, and mottled. They contain a single, hard-shelled seed with a reticulate pattern, protected by the thin wall of the ovary, displaying a tortoiseshell characteristic on the cannabis seed surface (United Nations Office on Drug and Crime, 2022a) (Figure 1).

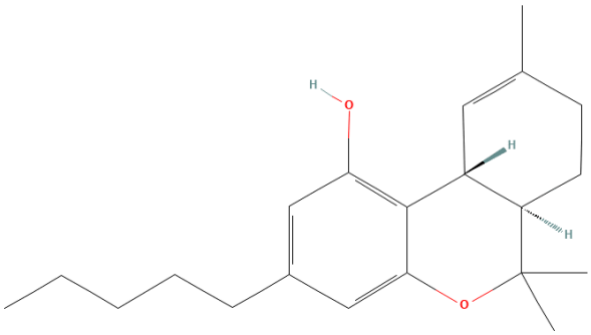
(United Nations Office on Drug and Crime, 2022a)

Figure 1: Marijuana morphology

The cannabis plant is known for its psychotropic effects due to the presence of the main alkaloids, i.e., cannabinol (CBN), cannabidiol (CBD), and 9-tetrahydrocannabinol (THC) (Pitri Susanti, 2012). It also contains other terpenophenolic secondary metabolites, including C21 alkyl resorcinol units and monoterpenes (ElSohly *et al.,* 2017). The two most notable phytocannabinoids are the 9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the primary psychoactive component, while CBD has no psychoactive action. The cannabis plant also contains several other chemicals, including cannabigerol (CBG), cannabichromene (CBC), and cannabidivarin (CBDV) (Alves et al., 2020). These compounds have distinctive mass fragmentation patterns, serving as specific markers: CBN 295, 296, 238, and 310m/z; 9-THC 299, 314, 231, 271m/z; Cannabichromene (CBC) 231, 232, 174, and 187m/z; CBD 231, 232, 246, and 174m/z (United Nations Office on Drug and Crime, 2022a).

Dronabinol, or delta-9 tetrahydrocannabinol or 9-THC, has a partially hydrogenated benzofuran structure (Figure 2). Its physicochemical and pharmacological aspects resemble C21H30O2, with a molecular weight of 314.5 g/mol. In water, it exhibits a solubility of 2,8 mg/L at 23°C, while its solubility in alcohols such as ethanol

and acetone exceeds 1 g/mL. This compound is characterised by a melting point below 25°C and a boiling point of 200°C. It can bind to CB1 receptors (coupled through G protein and modulated by adenylate cyclase and ion channels) in the central nervous system and CB2. Its activity involves inhibiting neurotransmitter release, thereby increasing dopaminergic signals, which increases the risk of delusions and hallucinations (Hazekamp *et al.,* 2005).



(National Center for Biotechnology Information, 2023)

Figure 2: Dronabinol compounds

The psychotropic effects of 9-THC are dose-dependent. Doses of 2-5 mg induce a sedative effect that causes a sense of comfort and euphoria in adults. Doubling this dosage (4-10 mg) results in sensory disturbances and changes in the perception of space and time. When doses reach 20-40 mg, individuals may experience confusion and hallucinations, and at higher doses, symptoms such as nausea, dizziness, and speech disturbances may occur. Despite these effects, consciousness remains, but brain capacity is substantially decreased (Jacobs & Steiner, 2014).

The colour test is the first step in conducting a general presumptive screening. It is nonspecific and gives a simple positive result through observable colour changes upon adding reagents to the sample. Hence, confirmatory analysis is still needed. This study's colour test used Fast Blue B and Duquenois methods.

Gas chromatography-mass spectrometry (GC-MS) is one of the most widely used confirmatory analytical techniques for identifying narcotics. It combines GC and MS, offering high selectivity in identifying volatile compounds or components. Gas chromatography relies on the rate at which, in a glass-packed column or metal tube coated with the stationary phase, a chemical (in the gas phase) is propelled through the stationary phase by the mobile phase (the carrier gas). Capillary columns can be up to 30 m long, with an internal diameter of up to 0.25 mm in glass (often fused silica). The stationary phase, supported by the capillary column, has a film thickness ranging from 0.1 to 0.25 m. The gas flow typically varies from 1-2 mL/min (Honour, 2006).

Parameters of the validated method for qualitative analysis of common cannabinoids, including cannabinoid acids, through derivatisation are crucial for accurate results. The working principle of GC-MS is the evaporation of the sample in the inlet or injector heated from the GC, creating differences in retention time. The distribution (partition) of each component between the mobile phase (carrier gas) and the stationary phase to the MS determines the separation of the components (Sparkman *et al.,* 2011). The GC-MS instrument's requirements include the need for the mobile phase to act as an inert, pure, and easily obtained gas, such as helium, argon, nitrogen, or hydrogen. The sample must be volatile.

Methods

Material

The sample used in this study was cannabis flowers from the Centre of Drug Testing Laboratory, National Narcotics Board RI. The positive control consisted of a mixture of cannabis flower leaves and seeds, confirmed to contain dronabinol. Tobacco served as the negative control.

Tools

The tools in this study included glassware commonly used for preparation purposes, ultrasonic baths, and chromatographic instrumentation involving a GCMS-QP2020Nx-2030Nexis and HP-5MS capillary column (30 m x 0.25 mm x 0.26 M). The temperature ranged from -60°C to 325°C, with a maximum limit of 350°C.

Physical test

Physical tests were carried out by looking at the description of the samples obtained.

Colour test

Duquenois test

The sample, negative control, and positive control were each placed in a test tube, and then 1% vanilla and concentrated HCl were added, followed by chloroform. Colour changes were observed.

Fast blue test

The sample, negative, and positive control were individually placed on a drip plate. Fast blue salt was

then added, followed by 4N NaOH. The resulting mixture was observed for any colour changes.

Confirmation test with GC-MS

The cannabis flower sample, negative control, and positive control weighed 0.25 g. These samples were then dissolved in 5 ml of methanol in separate vials. All vials were then sonicated for 30 minutes. Afterwards, the solutions were filtered with a 0.25µm PTFE micro filter and put in a 1.5 ml GC-MS vial.

Samples, negative control, and positive control measuring 1 µL were injected into GC-MS using a micro syringe and directed into a capillary column. The analysis was conducted using a Shimadzu GCMS-QP2020Nx-2030Nexis. The initial column temperature was 50°C, then increased by 10°C per minute until reaching 200°C, which was held for 5 minutes. Subsequently, the temperature was further increased at 10°C per minute until reaching 300°C, which was maintained for an additional 5 minutes.

Results

Table I displays the results of the Duquenois test, and Table II shows the results of the Fast Blue test. Figure 3 illustrates the sample chromatogram. Table III provides the peak report of TIC.

Table I: Duquenois test results

	Before	After
Positive control	Brown	Purple bottom layer
Negative control	Brown	Brown
Sample	Brown	Purple bottom layer

Table II: Fast Blue test results

	Before	After
Positive control	Brown	Brick red/peach
Negative control	Brown	Brown
Sample	Brown	Brick red/peach

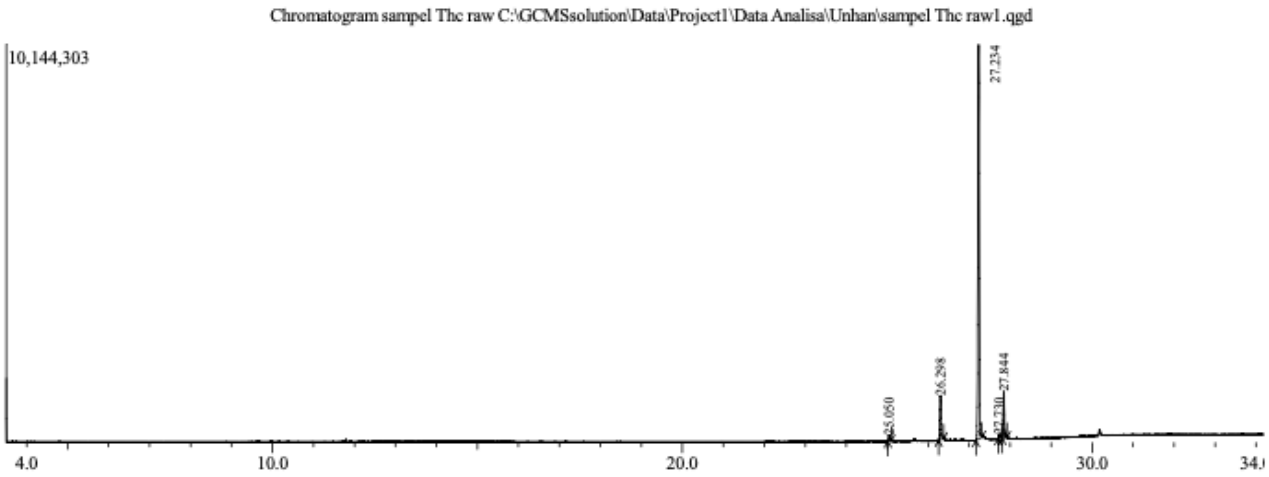


Figure 3: Sample chromatogram

Table III: Peak report TIC

Peak#	R.time	Area	Area %	Height	Height %	Similarity	Name
1	26.297	1495268	5.48	688518	5.46	98	Cannabichrome
2	27.230	20198429	74.05	9298772	73.78	97	Dronabinol
3	27.841	5584441	20.47	2616473	20.76	98	Cannabinol
		27278138	100.00	12603763	100.00		

Figure 4 displays a chromatogram overlaying the sample with the positive control for comparison. Table IV presents the identification of the sample compound result.

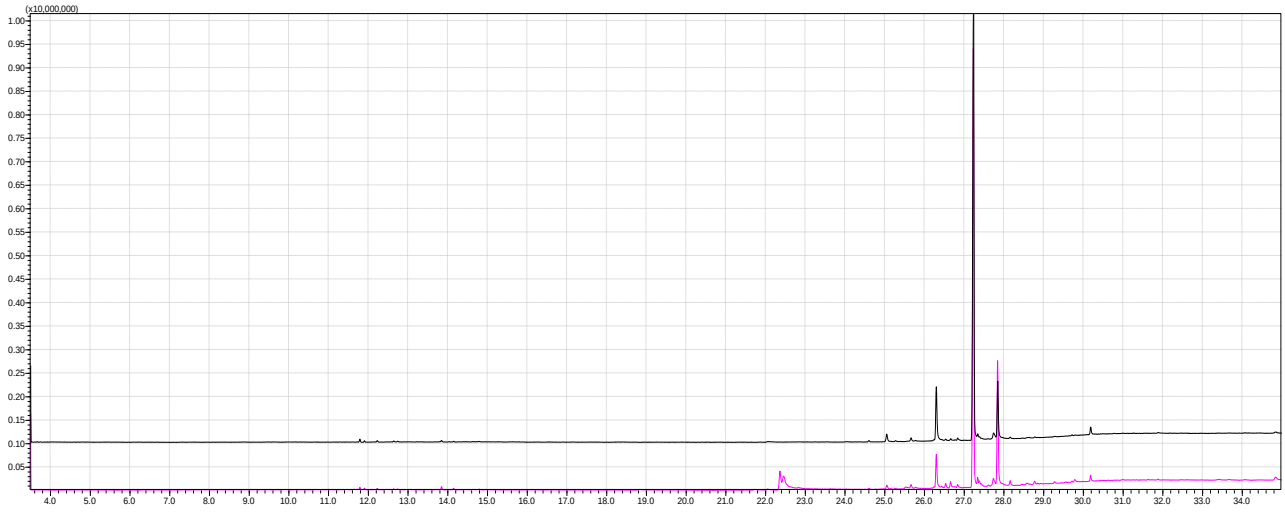


Figure 4: Overlay chromatogram compared sample with the positive control

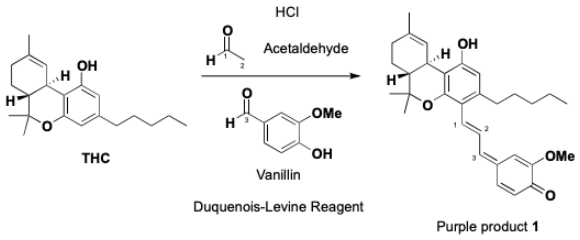
Table IV: Sample compound result identification

No	Compound name	Mass fragmentation	Retention time	Peak (%)	Area	BM	Library
1.	Δ9-Tetrahidrocannabivarin	203, 243, 271, 286 m/z	20.05	1.18	323970	286	NIST
2.	Cannabichromene	174, 231, 314, m/z	26.30	8.89	2491240	314	NIST
3.	Dronabinol	231, 271, 299, 314 m/z	27.23	79.75	22807585	314	NIST
4.	Cannabigerol	123, 193, 207, 231 m/z	27.74	0.71	316506	316	NIST
5.	Cannabinol	238, 295, 310 m/z	27.85	9.47	2472410	310	NIST

Discussion

Cannabis sample colour test

Identification of 9-THC compounds in samples suspected to contain marijuana involved two stages, i.e. screening tests and confirmation tests. The screening began with a physical examination to confirm that the description meets that of the cannabis plant. Colour tests were then carried out using the Dequenois (Figure 5) and Fast Blue methods, followed by a confirmation test using GC-MS to validate the presence of 9-THC compounds in the test sample. After physical examination, two colour tests were conducted to compare the samples with negative and positive controls. The first performed test was the Duquenois colour test, employing 1% vanillin solution and 12N concentrated HCl as reagents. The principle of this test is the reaction between the sample and two reagents, vanillin and concentrated HCl, resulting in colour change. The two reagents were added to the samples, and the negative and positive controls were added, forming a blue colour indicating a positive result. Then, chloroform was added to form different layers. HCl served as an acid medium to activate THC compounds in the sample.



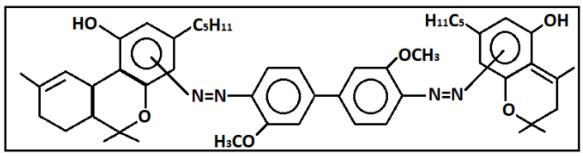
(Watanabe et al., 2017)

Figure 5: Duquenois reaction with THC

In this Duquenois test, 9-THC, 8-THC, CBD, and CBN will react with the reagents to produce a chromophore, resulting in a specific colour, serving as a positive marker (Watanabe *et al.,* 2017). The colour change is due to the reaction between THC in the cannabis sample and the Duquenois reagents (Jacobs & Steiner, 2014). After the colour change occurred, chloroform was added to separate the THC compound from dried cannabis leaves through solid-liquid extraction, marked by two layers, a blue top layer indicating the presence

of THC compounds and a purple bottom layer indicating chloroform.

The second test was the Fast Blue colour test, utilising Fast Blue salt reagents and 4N NaOH. The test relies on the principle of a red colour change, indicating the presence of 9-THC, CBD, and CBN compounds (Figure 6). The reaction between the Fast Blue B salt (Di-o-anisidinetetrazolium chloride) and the sample produces a red brick or peach, indicating a positive result. This method is used to identify THC in marijuana, hashish, hash oil, or fresh marijuana green leaf material (Acosta & Almirall, 2021). The Fast Blue salt was added to negative and positive control samples, followed by NaOH 4N. A positive result was indicated by the formation of a brick red or peach colour after the reaction.



(Acosta & Almirall, 2021)

Figure 6: Fast Blue reaction with THC

Cannabis sample confirmation test

A GCMS confirmation test was carried out with the sample, negative, and positive control.

To confirm the suspected cannabis plant samples, 9-THC compounds in cannabis leaves were identified by comparing the fragmentation pattern of the 9-THC mass spectrum with the fragmentation pattern of the literature. The obtained results revealed five peaks from the GC analysis, reinforced by the spectral data and the structure of the compound produced by MS.

The GC-MS analysis yielded two sets of data: chromatograms from gas chromatography (GC) analysis and mass spectra from mass spectroscopic (MS) analysis. The chromatogram revealed five peaks, with the main compound identified as dronabinol. The results of the mass spectra analysis were as follows:

• First peak (retention time: 20.05 minutes): Identified as 9-tetrahidrocannabivarin, with a peak intensity of 1.18%, an area of 323970, and a similarity of 91% based on NIST library readings.

• Second peak (retention time: 26.30 minutes): Identified as cannabichromene, with a peak intensity of 8.89%, an area of 2491240, and a similarity of 98% based on NIST library readings.

• Third peak (retention time: 27.23 minutes): Identified as dronabinol, with a peak intensity of 79.75%, an area of 22807585, and a similarity of 96% based on NIST library readings.

• Fourth peak (retention time: 27.74 minutes): Identified as cannabigerol, with a peak intensity of 0.71%, an area of 316506, and a similarity of 92% based on NIST library readings.

• Fifth peak (retention time: 27.85 minutes): Identified as cannabinol, with a peak intensity of 9.47%, an area of 2472410, and a similarity of 98% based on NIST library readings.

Conclusion

Based on the results of the screening tests (Duquenois and Fast Blue Salt colour tests), the analysed samples were suspected to contain marijuana. Confirmation testing using the GC-MS identified five compounds: 9-tetrahydrocannabivarin, cannabichromene, dronabinol, cannabigerol, and cannabinol, with dronabinol being the most intense compound. These five compounds are known cannabinoid compounds found in marijuana. Therefore, it can be concluded that the tested sample is indeed marijuana.

Acknowledgement

The authors would like to thank Dasuri, Lilik Hidayati, Tesia Aisyah Rahmania, Adellia Agatha, and Faculty of Military Pharmacy members for supporting them with this article.

References

Acosta, A., & Almirall, J. (2021). Differentiation between hemp-type and marijuana-type cannabis using the Fast Blue BB colorimetric test. *Forensic Chemistry,* **26**, 100376. https://doi.org/10.1016/j.forc.2021.100376

Alves, P., Amaral, C., Teixeira, N., & Correia-da-Silva, G. (2020). Cannabis sativa: Much more beyond Δ9-tetrahydrocannabinol. *Pharmacological Research,* **157**, 104822. https://doi.org/10.1016/j.phrs.2020.104822

Badan Narkotika Nasional. (2022). *BNN musnahkan 78,2 Kg sabu dan 61,9 Kg ganja*. https://bnn.go.id/bnn-musnahkan-782-kg-sabu-619-kg-ganja/

Chandra, S., Lata, H., & ElSohly, M. A. (Eds.). (2017). *Cannabis sativa L. - Botany and biotechnology.* Springer International Publishing. https://doi.org/10.1007/978-3-319-54564-6

ElSohly, M. A., Radwan, M. M., Gul, W., Chandra, S., & Galal, A. (2017). Phytochemistry of Cannabis sativa L. In A. D. Kinghorn, H. Falk, S. Gibbons, & J. Kobayashi (Eds.), *Phytocannabinoids: Unraveling the complex chemistry and pharmacology of Cannabis sativa* (pp. 1–36). Springer International Publishing. https://doi.org/10.1007/978-3-319-45541-9_1

Hazekamp, A., Peltenburg, A., Verpoorte, R., & Giroud, C. (2005). Chromatographic and spectroscopic data of cannabinoids from Cannabis sativa L. *Journal of Liquid Chromatography and Related Technologies,* **28**(15), 2361–2382. https://doi.org/10.1080/10826070500187558

Honour, J. W. (2006). Gas chromatography-mass spectrometry. In M. J. Wheeler & J. S. M. Hutchinson (Eds.), *Hormone assays in biological fluids* (pp. 53–74). Humana Press. https://doi.org/10.1385/1-59259-986-9:53

Isnaini, E. (2017). Penggunaan ganja dalam ilmu pengobatan menurut Undang-Undang Nomor 35 Tahun 2009 tentang narkotika. *Jurnal Independent,* **5**(2), 46. https://doi.org/10.30736/ji.v5i2.71

Jacobs, A. D., & Steiner, R. R. (2014). Detection of the Duquenois-Levine chromophore in a marijuana sample. *Forensic Science International,* **239**, 1–5. https://doi.org/10.1016/j.forsciint.2014.02.031

McPartland, J. M. (2018). Cannabis systematics at the levels of family, genus, and species. *Cannabis and Cannabinoid Research,* **3**(1), 203–212. https://doi.org/10.1089/can.2018.0039

National Center for Biotechnology Information. (2023). *PubChem compound summary for CID 16078, Dronabinol*.

Pitri Susanti, N. (2012). Identifikasi kandungan cannabinoid dalam ekstrak batang ganja dengan metode Al-Tlc dan Hptlc spectrophotodensitometry. *Indonesian Journal of Legal and Forensic Sciences,* **2**(1), 282193. https://ojs.unud.ac.id/index.php/ijlfs/article/view/3249

Puslitdatin BNN. (2022). *Indonesia drugs report 2022*. Pusat Penelitian, Data, Dan Informasi Badan Narkotika Nasional (PUSLITDATIN BNN) (p. 24).

Sparkman, O. D., Penton, Z. E., & Kitson, F. G. (2011). *Gas chromatography and mass spectrometry: A practical guide* (2nd ed.). Academic Press. https://doi.org/10.1016/B978-0-12-373628-4.00056-3

Thomas, B. F., & ElSohly, M. A. (2016). The botany of Cannabis sativa L. In *The analytical chemistry of Cannabis* (Issue December 2016). https://doi.org/10.1016/b978-0-12-804646-3.00001-1

Undang Undang RI. (2009). *Undang Undang RI 2009 tentang Narkotika.*

United Nations Office on Drug and Crime. (2022a). *Recommended methods for the identification and analysis of cannabis and cannabis products*. https://www.unodc.org/documents/scientific/Recommended_methods_for_the_Identification_and_Analysis_of_Cannabis_and_Cannabis_products.pdf

United Nations Office on Drug and Crime. (2022b). *World drug report 2022*. https://www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2022.html

Watanabe, K., Honda, G., Miyagi, T., & Kanai, M. (2017). The Duquenois reaction revisited: Mass spectrometric estimation of chromophore structures derived from major phytocannabinoids. *Forensic Toxicology,* **35**, 185–189. https://doi.org/10.1007/s11419-016-0337-6