HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

http://dx.doi.org/10.1016/j.apjtm.2017.03.011

Determination of delta-9-tetrahydrocannabinol content of cannabis seizures in Egypt

Ahmed M.A. Souleman[1], Alaa El-Din M. Gaafar[2], Omar M. Abdel-Salam[3]✉, Shaimaa A. ElShebiney[3]

[1]*Department of Phytochemistry and Plant Systematic, National Research Centre, Cairo, Egypt*

[2]*Department of Photochemistry, National Research Centre, Cairo, Egypt*

[3]*Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 20 Nov 2016
Received in revised form 21 Dec 2016
Accepted 20 Jan 2017
Available online 6 Mar 2017

Keywords:
Cannabis
Hashish
Marijuana
THC
High performance liquid chromatography

ABSTRACT

Objective: To determine the delta-9-tetrahydrocannabinol (THC) content of cannabis seizures in Egypt.
Methods: Unheated and heated extracts of cannabis seizures were prepared from the dried flowering tops and leaves (marijuana) or from the resin (hashish) and subjected to analysis using high performance liquid chromatography (HPLC).
Results: The heated resin extract had the peak of THC in a relative ratio of 31.34%, while extracting the resin directly without heating contained only 18.34% of THC. On the other hand, marijuana showed minimum percentage of THC at 11.188% on heating and 9.55% without heating.
Conclusions: These results indicate the high potency of the abused cannabis plant in the illicit Egyptian market.

1. Introduction

Cannabis (from the female plant of *Cannabis sativa* L; family *Cannabidaceae*) is the major trafficked drug across the world according to the 2014 World Drug Report [1], and the most abused drug in Egypt [2]. About 2.7%–4.9% of the population aged (15–64) years have used cannabis at least once in their life time [1]. It is prevalent among the Egyptian youth and drivers for its recreational properties and the belief it is harmless [3]. Seizures usually presented to the criminal laboratories are either the raw plant material itself (marijuana) or the resin of the cannabis plant (hashish). Marijuana seizures, commonly referred to as 'bango', are either internally cultivated in Egypt or externally imported. However, hashish is generally imported from different countries and smuggled across the northern coasts or western margins [4,5]. Cannabis contains over 400 compounds [6], of which the Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN) are the most common [7]. The psychotropic effects of cannabis are mediated by THC. The latter is the main constituent in herbal cannabis and hashish [8]. However, CBD and CBN have gained attention as they have pharmacological properties in addition the former might antagonize some of the effects produced by THC [9].

Determination of cannabis constituents is of great value both to authorities as well as researchers as there is evidence of changing THC concentration and consequently psychotropic potency across time since the eighties and nineties [10,11]. Up to our knowledge, there are no research reports from Egypt discussing the potency of abused cannabis, despite of its abundance and popularity among drug abusers in Egypt. Therefore, this study aimed to investigate the available market products to explore the potency trend in the local market and shed the light for medical guidance in Egypt.

2. Material and methods

2.1. Cannabis seizures and chemicals

Cannabis seizures of either the herb (marijuana) or the resin (hashish) were obtained officially from Forensic chemistry

First author: Ahmed M. A. Souleman, Department of Phytochemistry and Plant Systematic, National Research Centre, Cairo, Egypt.
✉Corresponding author: Omar M.E. Abdel Salam, Department of Toxicology and Narcotics, National Research Centre, Tahrir St., Dokki, Cairo, Egypt.
Fax: +20 2 33370931
E-mail: omasalam@hotmail.com
Peer review under responsibility of Hainan Medical University.
Foundation Project: This works is supported by NRC (grant number 10001004).

laboratories, Ministry of Justice, Egypt. The seizures were confirmed morphologically using a simple light microscope and preliminary color test. Standard THC was a generous gift from the Forensic Labs of Ministry of Justice, Egypt. The organic solvents used were of high performance liquid chromatography (HPLC) or analytical grade and purchased from Sigma (St. Louis, MO, USA).

2.2. Extraction of cannabis

Dry extract of cannabis seizures was prepared from the dried flowering tops and leaves (marijuana) or from the resin (hashish). The method of extraction followed that described by Turner and Mahlberg [12] with modification. In brief, 10 g of each sample of dried cannabis (marijuana or hashish) was divided into small pieces, well-grounded, either heated in a glass baker in boiling water at 100 °C for 2 h [13] or used without heating. Then, unheated and heated samples were extracted with chloroform overnight to yield 2 g of dry extract and were protected from light and heat (stored at 4 °C, and protected from light by placing in an aluminum-covered container). This provided the dry extract as residue. The residue of unheated and heated marijuana or hashish extracts was then subjected to the analytical procedures.

2.3. Analysis of cannabis constituents

2.3.1. Column chromatography

Two grams of dry extract residue were re-dissolved in 20 mL methanol. Twenty gram of silica gel was added to the prepared solution and the whole mixture was evaporated in vacuum to ensure the adsorption of THC on surface of silica gel. The solid mixture was placed on a top of 200 g of silica gel in chloroform column separated with a layer of sea-sand. Another sea-sand layer was placed on the top of the column. The column was eluted with chloroform–methanol (5%). The fractions were examined by TLC and those that contained the same compound were collected together, evaporated in vacuum, each fraction was tested with a specific test for cannabinoids (Fast Blue B salt). The fractions that showed positive reaction were further identified by ^{1}H-NMR. The pure cannabinoid compound collected, weighed (yield 0.3 g), and the TLC indicates that there is only one cannabinoid compound.

2.3.2. High performance liquid chromatography

The dried residue was reconstituted in 2 mL chloroform and filtered over membrane filter (Whatman, pore size 0.45 μm) then 5 μL were injected manually. Standard THC was prepared in methanol and filtered over membrane filter (Whatman) and 5 μL were injected. HPLC profiles were acquired on a Schimadzu SCL-10Avp (Japan). HPLC system consisting of LC-10AD vp pump, DGU-14A degasser, and UV–vis (SPD-10A vp) detector. Full spectra were recorded in the range of (200–400) nm. A LiChrospher RP-18 analytical column (4.0 × 250.0 mm, 5 μL) was used. The mobile phase consisted of 2.5 M sulphuric acidified water:acetonitrile (17:83), pH 1.8 at isocratic elution. The column was re-equilibrated under initial conditions for 4 min. Flow rate was 1 mL/min and total runtime was 60 min. All determinations were carried out at ambient temperature.

3. Results

3.1. Column chromatography

Fractions collected at MeOH:$CHCl_3$ gradient (1:9) contained purely THC that was identified by Fast Blue B color test. Those fractions were collected and served as pure reference material in the proceeding analysis.

3.2. High performance liquid chromatography

The THC standard profile showed THC peak at Rt 8 min. The heated resin extract revealed the peak of THC in a relative ratio of 31.34%, while extracting the resin directly without heating contained only 18.34% of THC. On the other hand, marijuana leaves showed minimum percentage of THC at 11.188% on heating and 9.550% without heating. Charts are displayed in Figure 1.

4. Discussion

In this study, seized samples of marijuana and hashish were extracted and its THC content was determined using HPLC. The results indicated a high content of THC in the sampled plant material reaching 30% in the heated resin and 11% in the heated marijuana. Cannabinoids are the main bioactive constituents found in the plant cannabis. Over 80 of these terpenophenolic compounds have been identified so far [14] with the most important one being THC, the ingredient that mediates the psychotropic properties of recreational cannabis [8]. The highest concentrations of cannabinoids can be found in the flowering tops and in the plant resin [15]. Cannabinoids exist in the plant cannabis as carboxylic acids and these are converted by heating into their corresponding neutral acids. Decarboxylation (loss of CO_2) of delta-9-tetrahydrocannabinolic acid (THCA) achieved by heating will thus result in the formation of THC. Further degradation of THC will result in CBN or delta-8-THC [16,17]. The higher content of THC in the heated compared with the unheated plant material is thus an expected finding. The cannabinoid content of the resin is also higher than that of the flowering tops and leaves [15]. In this study, the highest concentrations of THC are thus found in the heated resin and marijuana extracts. Decarboxylation was found to be best at 100 °C for 2 h [13] which was implicated in the present study. Chloroform is also known to be a suitable solvent for the separation of THC from the other major neutral cannabinoids, such as CBC and CBD [13].

In this study, cannabis resin or hashish that is available on the illicit market in Egypt has shown a great potency compared to the worldwide reported concentrations of THC. In addition, the most widely abused form, marijuana, has shown a higher THC content than we expected based on the world drug reports. Several studies reported marked increase in THC content of the cannabis plants and resins over the past decade [18]. ElSohly *et al.* [11] found that the potency (concentration of THC) of illicit marijuana samples in USA increased from <1.5% in 1980 to 3.3% in 1983 and 1984 reaching 4.2% in 1997. Stefanidou *et al.* [19] reported THC concentrations that ranged from 0.08% to 4.41% in samples of illicit herbal cannabis in Greece. The reported THC content of herbal cannabis and

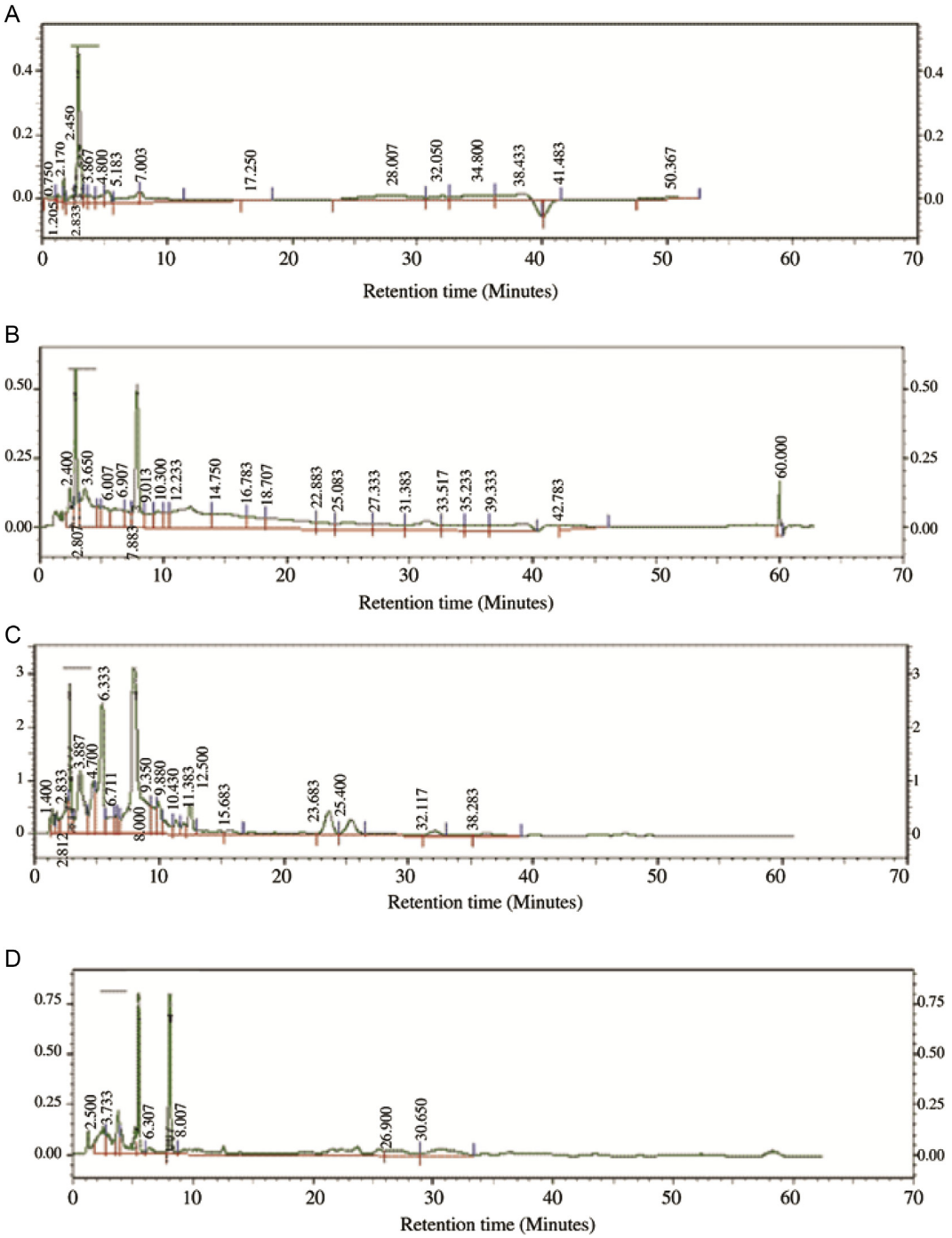

Figure 1. HPLC charts of heated cannabis resin extract, unheated resin extract, heated marijuana extract and unheated marijuana extract.
(A) Heated resin extracts showing the peak of THC at Rt 7.9 and resembling 31% of total peaks. (B) Unheated cannabis resin extract showing the peak of THC at Rt 7.88 and resembling 18% of total peaks. (C) Heated marijuana extract showing the peak of THC at Rt 8 and resembling 11% of total peaks. (D) Unheated marijuana extract showing the peak of THC at Rt 8 and resembling 9.5% of total peaks.

resin seized in England in 2004/2005 was 2.1% and 3.5%, respectively. The median THC content of the indoor-grown unpollinated female cannabis ('sinsemilla') was 13.9% [20]. An analysis of cannabis seizures in Australia showed high mean THC + THCA content of 14.88% [21]. The highest THC concentrations reported were that of cannabis resin made from Dutch marijuana (Nederhasj) that contained 30.0% THC (compared with 18.2% in imported hashish) [12,22]. On the other hand, Zamengo *et al.* [23] found large variation in THC content of samples of cannabis products analyzed during 2010–2012 in Venice area (Italy). The THC content, ranged from 0.3% to 31% for cannabis resin and from 0.1 to 19% for herbal cannabis. The increase in THC content of cannabis preparations can be explained by the introduction of hybrids with higher potency [24].

Cannabis abuse is a worldwide problem that seems on the rise [25–27]. Studies indicate increased cannabis use [27] and increased emergency department visits for the users of cannabis-only and cannabis-polydrug [28]. THC is known or its negative impact on the motor and cognitive functions e.g., verbal learning, memory, attention, risk taking behavior and psychological disorders, such as schizophrenia [29–31].

Increased THC content in available illicit cannabis could thus imposes a risk for driving problems and psychological health.

Conflicts of interest statement

The authors declare that there are no competing conflicts of interest.

References

[1] United Nations Office on Drugs and Crime (UNODC). *World drug report*. Vienna, Austria: United Nations Publications; 2014.
[2] Okasha A. Focus on psychiatry in Egypt. *BJP* 2004; **185**: 266-272.
[3] Hamdi E, Gawad T, Khoweiled A, Sidrak AE, Amer D, Mamdouh R, et al. Lifetime prevalence of alcohol and substance use in Egypt: a community survey. *Subst Abus* 2013; **34**(2): 97-104.
[4] United Nations Office on Drugs and Crime (UNODC). *World drug report*. Vienna, Austria: United Nations Publications; 2012.
[5] United Nations Office on Drugs and Crime (UNODC). *World drug report*. Vienna, Austria: United Nations Publications; 2013.
[6] ElShohly MA. Chemical constituents of cannabis. In: Grotenhermen F, Russo E, editors. *Cannabis and cannabinoids. Pharmacology, toxicology and therapeutic potential*. New York: Haworth Press Inc; 2002, p. 27-36.
[7] Pertwee RG. Pharmacological actions of cannabinoids. *Handb Exp Pharmacol* 2005; **168**: 1-51.
[8] Mechoulam R, Gaoni Y. The absolute configuration of delta-1-tetrahydrocannabinol, the major active constituent of hashish. *Tetrahedron Lett* 1967; **12**: 1109-1111.
[9] Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol* 2008; **153**(2): 199-215.
[10] ElSohly MA, Ross SA, Mehmedic Z, Arafat R, Yi B, Banahan BF 3rd. Potency trends of delta9-THC and other cannabinoids in confiscated marijuana from 1980–1997. *J Forensic Sci* 2000; **45**(1): 24-30.
[11] Pijlman FT, Rigter SM, Hoek J, Goldschmidt HM, Niesink RJ. Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. *Addict Biol* 2005; **10**(2): 171-180.
[12] Turner JC, Mahlberg PG. Separation of acid and neutral cannabinoids in *Cannabis sativa* L. using HPLC. In: Agurell S, Dewey WL, Willete RE, editors. *Pharmacology of therapeutic agents*. USA: Academic Press; 1984, p. 79-88 (in italic).
[13] Fischedick JT, Glas R, Hazekamp A, Verpoorte R. A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in *Cannabis sativa* L. *Phytochem Anal* 2009; **20**(5): 421-426.
[14] Radwan MM, Ross SA, Ahmed SA, Slade D, Zulfiqar F, ElSohly MA. Isolation and characterization of new cannabis constituents from a high potency variety. *Planta Med* 2008; **74**(3): 267-272.
[15] Ashton CH. Pharmacology and effects of cannabis: a brief review. *Br J Psychiat* 2001; **178**: 101-106.
[16] Eichler M, Spinedi L, Unfer-Grauwiler S, Bodmer M, Surber C, Luedi M, et al. Heat exposure of *Cannabis sativa* extracts affects the pharmacokinetic and metabolic profile in healthy male subjects. *Planta Med* 2012; **78**(7): 686-691.
[17] Hazekamp A. An evaluation of the quality of medicinal grade cannabis in the Netherlands. *Cannabinoids* 2006; **1**(1): 1-9.
[18] Cascini F, Aiello C, Di Tanna G. Increasing delta-9-tetrahydrocannabinol (Δ-9-THC) content in herbal cannabis over time: systematic review and meta-analysis. *Curr Drug Abuse Rev* 2012; **5**(1): 32-40.
[19] Stefanidou M, Athanaselis S, Alevisopoulos G, Papoutsis J, Koutselinis A. Delat9-tetrahydrocannabinol content in cannabis plants of Greek origin. *Chem Pharm Bull (Tokyo* 2000; **48**(5): 743-745.
[20] Potter DJ, Clark P, Brown MB. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *J Forensic Sci* 2008; **53**(1): 90-94.
[21] Swift W, Wong A, Li KM, Arnold JC, McGregor IS. Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PLoS One* 2013; **8**(7): e70052.
[22] Niesink RJ, Rigter S, Koeter MW, Brunt TM. Potency trends of Δ9-tetrahydrocannabinol, cannabidiol and cannabinol in cannabis in the Netherlands: 2005–15. *Addiction* 2015; **110**(12): 1941-1950.
[23] Zamengo L, Frison G, Bettin C, Sciarrone R. Variability of cannabis potency in the Venice area (Italy): a survey over the period 2010–2012. *Drug Test Anal* 2014; **6**(1–2): 46-51.
[24] Chouvy PA, Afsahi K. Hashish revival in Morocco. *Int J Drug Policy* 2014; **25**(3): 416-423.
[25] Botoeva G. Hashish as cash in a post-Soviet Kyrgyz village. *J Drug Policy* 2014; **25**(6): 1227-1234.
[26] Choi NG, DiNitto DM, Marti CN. Older marijuana users: life stressors and perceived social support. *Drug Alcohol Depend* 2016; **169**: 56-63.
[27] Lanza ST, Vasilenko SA, Dziak JJ, Butera NM. Trends among U.S. high school seniors in recent marijuana use and associations with other substances: 1976–2013. *J Adolesc Health* 2015; **57**(2): 198-204.
[28] Zhu H, Wu LT. Trends and correlates of cannabis-involved emergency department visits: 2004 to 2011. *J Addict Med* 2016; **10**(6): 429-436.
[29] Broyd SJ, van Hell HH, Beale C, Yücel M, Solowij N. Acute and chronic effects of cannabinoids on human cognition – a systematic review. *Biol Psychiatry* 2016; **79**(7): 557-567.
[30] Frascarelli M, Quartini A, Tomassini L, Russo P, Zullo D, Manuali G, et al. Cannabis use related to early psychotic onset: role of premorbid function. *Neurosci Lett* 2016; **633**: 55-61.
[31] Lisdah KM, Wright NE, Medina-Kirchner C, Maple KE, Shollenbarger S. Considering cannabis: the effects of regular cannabis use on neurocognition in adolescents and young adults. *Curr Addict Rep* 2014; **1**: 144-156.