Forensic Science International 222 (2012) 40–46

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

First systematic evaluation of the potency of *Cannabis sativa* plants grown in Albania

Zana Bruci [a], Ioannis Papoutsis [b], Sotirios Athanaselis [b], Panagiota Nikolaou [b], Ermira Pazari [a], Chara Spiliopoulou [b], Gentian Vyshka [c,*]

[a] Department of Forensic Medicine and Toxicology, School of Medicine, University of Tirana, Albania
[b] Department of Forensic Medicine and Toxicology, School of Medicine, National and Kapodistrian University of Athens, Greece
[c] Faculty of Medicine, University of Tirana, Albania

ARTICLE INFO

Article history:
Received 20 November 2011
Received in revised form 18 April 2012
Accepted 30 April 2012
Available online 18 May 2012

Keywords:
Cannabis sativa
D9-tetrahydrocannabinol
Cannabinoid content
GC–MS analysis

ABSTRACT

Cannabis products (marijuana, hashish, cannabis oil) are the most frequently abused illegal substances worldwide. Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component of *Cannabis sativa* plant, whereas cannabidiol (CBD) and cannabinol (CBN) are other major but no psychoactive constituents. Many studies have already been carried out on these compounds and chemical research was encouraged due to the legal implications concerning the misuse of marijuana. The aim of this study was to determine THC, CBD and CBN in a significant number of cannabis samples of Albanian origin, where cannabis is the most frequently used drug of abuse, in order to evaluate and classify them according to their cannabinoid composition. A GC–MS method was used, in order to assay cannabinoid content of hemp samples harvested at different maturation degree levels during the summer months and grown in different areas of Albania. This method can also be used for the determination of plant phenotype, the evaluation of psychoactive potency and the control of material quality. The highest cannabinoid concentrations were found in the flowers of cannabis. The THC concentrations in different locations of Albania ranged from 1.07 to 12.13%. The influence of environmental conditions on cannabinoid content is discussed. The cannabinoid content of cannabis plants were used for their profiling, and it was used for their classification, according to their geographical origin. The determined concentrations justify the fact that Albania is an area where cannabis is extensively cultivated for illegal purposes.

1. Introduction

Cannabis is considered as the most controversial plant worldwide, next to the important medical use, and it is also the most frequently consumed drug of abuse in European countries [1]. Cannabis products can be consumed in a variety of ways, such as smoking, vaporizing, preparing cannabis tea and using it in baked products [2].

The use of cannabis products (e.g. marijuana, hashish), particularly among the young generations, is increased lately and it has been studied in many epidemiological surveys [3]. Marijuana continues to be the most readily available drug of abuse in Albania and the number and size of marijuana seizures have also been increased. Since nineteen's, Albania has become the major supplier of cannabis to other countries, such as Greece and Italy [4]. Following large societal concerns and neighbor countries' pressure, very restrictive policies have been put in force from the Albanian legislative power; since 2004 only the industrial hemp can be cultivated in Albania, and the fiber-type seeds should not exceed an amount of 0.1% of tetrahydrocannabinol, according to the actual law [5].

Delta-9-tetrahydrocannabinol (THC) is one of the major cannabinoids in *Cannabis sativa* and it has been proven to be the compound that possesses the psychoactive properties [6]. The endocannabinoid system includes two receptors, CB_1 and CB_2; and possibly others, expressed in the central nervous system, in the peripheral nervous system as well in a variety of circulating immune cells [7]. An oral dose of 20 mg of THC produces effects of mood, memory, motor coordination, cognitive ability, sensorium and self-perception; most commonly there is an increased sense of well-being or euphoria, followed from relaxation and sleepiness [8]. Rewarding effects and equivalents of a withdrawal syndrome are described, particularly with behavioral manifestations [9].

* Corresponding author at: Faculty of Medicine, University of Tirana, Rr. Dibres 371, Tirana, Albania. Tel.: +355 672907082.
E-mail addresses: zbruci@yahoo.com (Z. Bruci), jopamal@hotmail.com (I. Papoutsis), sathan@med.uoa.gr (S. Athanaselis), pan_nik@hotmail.com (P. Nikolaou), emi.pazari@gmail.com (E. Pazari), chspiliop@med.uoa.gr (C. Spiliopoulou), gvyshka@yahoo.com (G. Vyshka).

0379-0738/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2012.04.032

Albeit the addictive properties are consistent, an increasingly amount of research has been dedicated to the therapeutic aspects of cannabis [10]. Thus, nausea and vomiting, multiple sclerosis, neurodegenerative diseases and depression, among others, have been targeted as possible conditions for a medically justified application of cannabis [10,11].

Non active cannabinoids tend to be present in significant amounts, including cannabinol (CBN) and cannabidiol (CBD). The concentrations of each cannabinoid can vary depending on genetic factors, environmental conditions of the cultivation and the time of harvest, or other factors such as drying and storage conditions [12].

THC is thermolabile and photolabile and as a result the storage of cannabis leads to a cumulative decrease in THC content through oxidation of THC to CBN [13].

On the basis of THC content, *C. sativa* plants are divided into fiber-type and drug-type [14]. Hillig and Mahlberg [15] identified three chemotypes of cannabis: drug-type plants (chemotype I) show a high ratio [total THC/total CBD] (>1.0), intermediate-type plants (chemotype II) have an intermediate ratio (close to 1.0), and fiber-type plants (chemotype III) exhibit a low ratio (<1.0). The phenotypic index (%THC + %CBN)/%CBD was also defined by Fetterman et al. [16]. If phenotypic index exceeds 1.0, the plant is classified as phenotype I, which represents the drug-cannabis, while if phenotypic index is less than 1.0 the plant is classified as phenotype II, which represents the non-drug cannabis [17]. For forensic and legal purposes a cannabis plant is generally considered as drug-type when its leaves and inflorescences present THC content not lower than 0.5% [18].

The cultivation of drug-type hemp is prohibited in several countries; since 2001 the European Union allows cultivation of fiber-type hemp varieties with THC content less than 0.2% [12]. The drug-type plant usually contains up to 5% of THC, though higher percentages (up to 10%) have been reported. It is widely accepted that the presence only of THC in substantial quantities (0.3%) qualifies a sample of cannabis as a drug, since CBD or CBN may be absent even from fresh samples [19]. Because that delta-9-tetrahydrocannabinol concentration decreases over time in cannabis plants, it will be difficult to distinguish between a fresh fiber cannabis sample and an old resin cannabis sample [19]. In some cannabis products the low proportion of THC is not incompatible with their resinous character, as CBN (a degradation product of THC) in these samples is presented in large proportions. This leads to the suggestion that THC plus CBN content in fact reflects the total THC content, irrespective of degradative changes [4].

For the characterization of the cannabis and the determination of its quality it is necessary to determine concentrations of THC, CBN and CBD. A selective and specific analytical method to determine different cannabinoids in one single run is needed for this reason. Previously published studies have shown that THC, CBD and CBN can be separated and potentially quantified using various TLC methods [20,21]; however, these methods require the use of over-pressured layer chromatography instrumentation or automated multiple development systems. Gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometer (MS) or other detectors [6,12,13,18,22–25] are the main techniques used for determination of cannabinoids contents in cannabis products.

The aim of this study was to determine THC, CBN and CBD in a significant number of cannabis samples that were cultivated in many areas of Albania during the last 3 years. Statistical analysis of the differences in the mean THC concentrations from year to year was carried out to ascertain the trend in the change in marijuana potency over time. Leaf content of cannabinoids at different times of the life cycle and also of plants coming from 11 areas of Albania and grown in a similar environment was examined. In this way, we tried to determine if the distinction of the two chemotypes described above can occur in any area of Albania or in any given developmental stage of the plant. This is extremely useful as the possession of the drug type of *C. sativa* is prohibited in most countries and the legal evaluation demands a rigorous and unequivocal means of identifying plant material, as drug or non-drug type, even when immature specimens are seized [26]. According to the same authors (Barni-Comparini et al.) each plant contains a predominant cannabinoid derivative that characterizes it throughout the whole period of growth and can be used reliably for forensic application concerning drug-suspected material in very young plants [26].

The present work is tending to monitor the physical and chemical characteristics of hemp plants grown in Albania. The combined results may provide the bases to enhance agronomic potential of hemp in compliance with law enforcement purposes. The paper describes also the GC–MS procedure, used to analyze the main cannabinoids in a considerable number of cannabis samples from different origin and age. The procedure has been previously validated from several studies [4,17,18]. To our knowledge, this is the first effort to determine the cannabinoid content of cannabis products of Albanian origin.

2. Experimental

2.1. Chemicals and preparation of standard solutions

Cannabinoid reference standards for THC, CBD, CBN and tetracosane were purchased from Sigma Aldrich (Steinheim, Germany) and all standards had purity higher than 98%.

All solvents used for the extraction procedure (toluene, hexane, methanol, ethyl acetate, ethyl ether and petroleum ether) were at least of analytical grade and were purchased from Merck (Darmstadt, Germany).

Ultrapure distilled and deionized water was prepared in-house and filtered prior to use with Milli-Q water purification system from Millipore (Kloten, Switzerland).

Stock standard solutions containing THC, CBD and CBN at a concentration of 1.0 mg/mL were prepared in methanol. Working solutions at concentration of 10.0 μg/mL were subsequently prepared by dilution of the stock standard solutions with methanol and stored at 4 °C until the analysis. The internal standard (I.S.) (tetracosane) working solution was prepared at a concentration of 50.0 μg/mL in methanol.

Calibrators containing THC, CBD and CBN at concentrations of 0.01, 0.05, 0.20, 1.00, 5.00 and 20.0% (w/w) and the three quality control samples with the three cannabinoids at concentrations of 0.03, 3.00 and 16.0% (w/w), were prepared daily for each analytical batch. Tubes with suitable amounts of methanol working solutions were evaporated under nitrogen before adding 10 mg of a pre-checked cannabinoid-free plant. Calibrators and quality control samples were treated and processed as unknown samples.

2.2. Plant material

2.2.1. The cultivation technique

The period of cultivation was between March and September. The cultivation was made by using seeds of Albanian origin. The seeds were not hybrids, but of the type *C. Sativa* subsp. sativa, collected from plants grown illegally in different Albanian regions. The planting has been made during March in seed beds at the same conditions and the plants were developed until the end of April. During May, the plants were transferred carefully outdoors at 11 different areas of Albania. They were planted at a distance of 1.5 m between each other and in straight lines. The watering of the plants was uniform. During June, blossoming was started and male and female plants were easily separated. The female plants had an average height of 1.6 m and green leaves, while the male plants had an average height of 0.8 m with light green leaves larger than the females. The plants were developed till the end of September when all the plants were harvested.

During the development of the plant different samples were collected; the number of samples was 10 (ten) for each analysis performed. Specifically we analyzed:

- Different parts of the female plants like flowers, leaves, seeds, stems and roots. These samples were harvested from several different plants during the flowering stage in August.
- Leaves and flowering tops from female plants during different vegetation periods. The leaves were collected after the 4th week of growth (April) and every month until September while the flowering tops were gathered after the 12th week of growth (June) and every month until September.
- Leaves from the same mature plants were collected during August at three different heights of the plants in the same day: (a) from 20 to 60 cm, (b) from 60 to100 cm and (c) above 100 cm.