IDENTIFICATION AND CHARACTERIZATION OF SPECIAL TYPES OF HERBAL CANNABIS

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Apariţia pe piaţa ilicită a drogurilor a unor noi soiuri hibride ale plantei de cannabis cu un potenţial psihoactiv ridicat necesită dezvoltarea continuă a unor metode adecvate de identificare şi caracterizare a acestora în scopul stabilirii zonelor de proveniență şi a rutelor de traficare a acestora. În cadrul acestei lucrări au fost identificate şi caracterizate din punct de vedere al conţinutului în canabinoizi, al fenotipului şi al vârstei mai multe soiuri hibride de canabis confiscate de către organele abilitate pe teritoriul României.

The appearance on the illicit drug market of new hybrid varieties of the cannabis plant with a high potency requires a continuous development of some appropriate methods for their identification and characterization in order to establish their arias of origin and trafficking routes. In this work it was identified and characterized from the point of view of content in cannabinoids, phenotype and age a series of hybrid varieties of cannabis seized by competent authorities in Romania.

Keywords: cannabis, cannabinoids, phenotype, potency

1. Introduction

Cannabis is one of the most used illicit drugs in UE. Generally, it is important to distinguish between different types of cannabis, especially when considering potency. Theoretically, a further distinction should be made whenever possible between imported herbal cannabis and home produced herbal cannabis [1].

The potency of cannabis is defined as the concentration of the major psychoactive component, namely Δ^9–tetrahydrocannabinol (Δ^9–THC), in the herbal cannabis. Although, sometimes could be found in the literature as

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"cannabis purity", this term is ambiguous and could refer to whether or not the material has been adulterated [2, 3].

Since CBN is almost entirely absent from fresh cannabis, the atmospheric exposure of Δ^9-THC causes its oxidation to cannabinol (CBN) decreasing the potency of cannabis. The rate of decomposition in cannabis at room temperature was estimated as 17% per year [4, 5]. Thus, CBN/Δ^9-THC ratio could serve as a measure of the age of herbal cannabis. Some imported products may have been harvested or manufactured months before consumption or analysis and, by contrast, local products will lead to a fresher product containing more Δ^9-THC [6].

Cannabis grown for fiber production (hemp) normally contains less than 0.3% Δ^9–THC and more cannabidiol (CBD). Cannabis grown for illegal traffic contains 2–8% Δ^9–THC. There are intermediate types which contain more than 0.5% Δ^9–THC and more than 0.5% CBD [7, 8].

Generally, there are two main types of cannabis that are grown for Δ^9–THC content, namely *indica* and *sativa*. *Cannabis indica* is characterized by broad, short leaves and heavy, tight flowers and has higher amount of CBD than Δ^9–THC. *Cannabis sativa* is characterized by long thin flowers and spiky leaves and has higher content of Δ^9–THC than CBD. Among many trials of breeding, crossing *sativa* and *indica* strains led to the development hybrids with high potency. The resulting hybrids strains will grow and mature in relationship with to the indica/sativa percentages. They appeared as new types of cannabis such as AK 47 and White Russian. Lab tests have rated the THC content at over 20%, making it a "*one hit wonder*" for many smokers. White Russian combines AK 47 with the White Widow to produce a snowy plant with a pleasantly sweet aroma and a strong, clear high cerebrally long lasting effect [9].

The objective of this paper is to identify and characterize, based on experimental results regarding the content of the major cannabinoids, phenotype and age of a new varieties of hybrid herbal cannabis seized by competent authorities in Romania.

2. Experimental

Materials, chemicals and reagents

Six types of cannabis samples seized by criminal prosecution authorities from Romania and provided by Central Laboratory for Drug Analysis and Profiling were subject to experimental investigation in this work. The cannabis types are as following: AK 47, Moby Dick, White Russian, AK Moon, Ice Bull, and Ro. The first five types of herbal cannabis have slang name while latter type has a name that indicate its provenience, namely Romania. The major differences

between them are the physical aspect and the content in the major component, namely tetrahydrocannabinol (Δ^9–THC).

All chemicals and reagents used for samples preparation and analysis were of analytical grade from Merck (Darmstadt, Germany). The etalons of major cannabinoids were purchased from Lipomed, Arlesheim, Switzeland.

Physical Examination

Herbal cannabis samples were identified by slang name based on their morphological characteristics by macroscopic and microscopic examination.

Samples preparation

The preparation of cannabis samples in order to be analyzed in terms of their major cannabinoids (Δ^9–tetrahydrocannabinol or Δ^9–THC, cannabidiol or CBD, cannabinol or CBN, cannabigerol or CBG, cannabichrome or CBC, Δ^9 – tetrahydrocannabivarin or THCV, and Δ^8–tetrahydrocannabinol or Δ^8–THC) is briefly described in the following. Thus, 0.2 g of dryed, homogenized and grinded herbal cannabis was extracted with 20 mL of methanol for 15 minutes in an ultrasonic bath. An extract aliquot was then transferred into a 2 ml GC vial. After this, the vial was put into a heating unit at 150°C for 12 minutes in order to evaporate the solvent and decarboxylate the tetrahydrocannabinolic acid (THCA). The obtained residue was extracted in methanol and then the resulted solution was analyzed by GC-MS (Gas Chromatography–Mass Spectrometry) and GC–FID (Gas Chromatography–Flame Ionization Detector).

Analytical methods

GC–MS analyses were carried out on a 6890N gas chromatograph coupled to a 5973N mass selective detector (Agilent, Waldbronn, Germany) and a CTC Combi-PAL autosampler (Chromtech, Idstein, Germany). Separation was achieved on a fused silica capillary column (HP–5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Temperature program: 150°C hold for 1 min, 10°C/min to 280°C, hold for 5 min. The injection port and interface temperature were 290°C and 300°C, respectively. Splitless injection mode was used and helium, with a flow rate of 1.0 mL per minute, was used as carrier gas. The scan range of MS (*m/z*) was in the range 40–450 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

GC–FID analyses were carried out on a 7890A gas cromatograph with a flame ionization detector (Agilent, Waldbronn, Germany). Separation was achieved on a fused silica capillary column (HP-5MS, 30 m × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Temperature program: 150°C hold for 1 min, 10°C/min to 280°C, hold for 5 min. The injection port and interface temperature were 250°C and 300°C, respectively. Split injection mode

was used (20:1) and hydrogen, with a flow rate of 30 mL per min, was used as carrier gas.

3. Results and discussion

Identification of major cannabinoids in herbal cannabis

Based on the above sample preparation method and GC–MS analysis the major cannabinoids in herbal cannabis was identified. The results are presented in Table 1 and gas-chromatographic profile of cannabis Moby Dick is shown in Fig. 1. Mass spectra of the most abundant cannabinoids identified in herbal cannabis (Δ^9–THC, CBD and CBN) are presented in Fig. 2.

Table 1

Major cannabinoids identified in herbal cannabis

Identified cannabinoid	Cannabis type					
	AK 47	***Moby Dick***	***White Russian***	***AK Moon***	***Ice Bull***	***Ro***
	Retention time, min					
Δ^8 –Tetrahydrocannabinol (Δ^8–THC)	-	11.17	-	-	-	11.15
Δ^9 –Tetrahydrocannabivarin (THCV)	11.49	11.49	11.49	11.50	-	11.47
Cannabichromene (CBC)	12.38	12.37	12.37	12.39	12.38	-
Cannabidiol (CBD)	-	-	-	-	-	12.41
Δ^9 –Tetrahydrocannabinol (Δ^9–THC)	13.23	13.24	13.23	13.24	13.23	13.16
Cannabigerol (CBG)	13.61	13.55	13.57	13.60	13.57	13.52
Cannabinol (CBN)	13.70	13.68	13.69	13.71	13.69	13.60

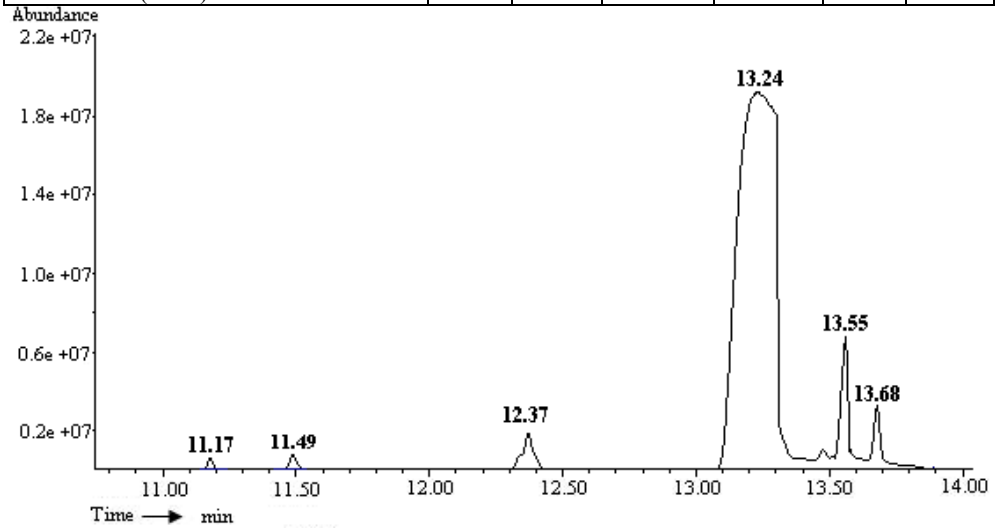
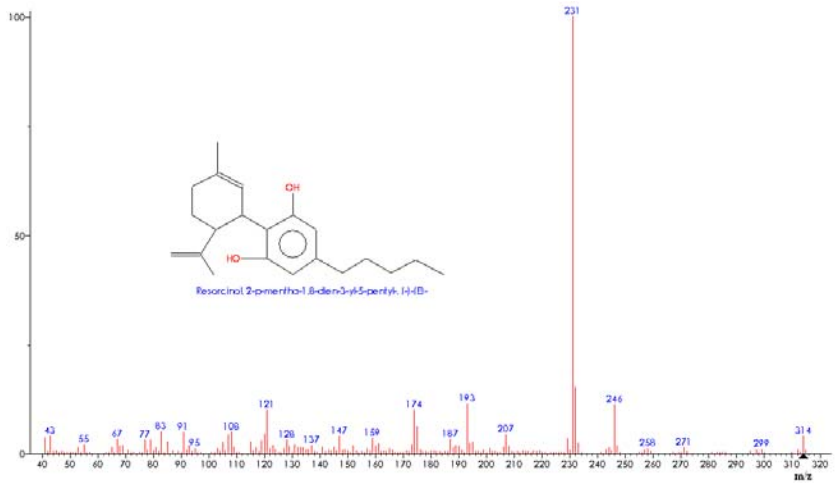
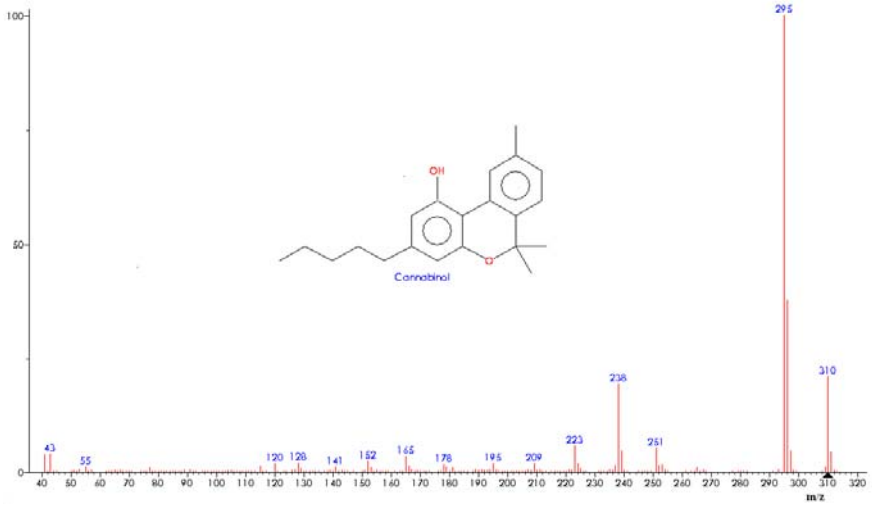


Fig. 1. GC-MS chromatogram of cannabis Moby Dick

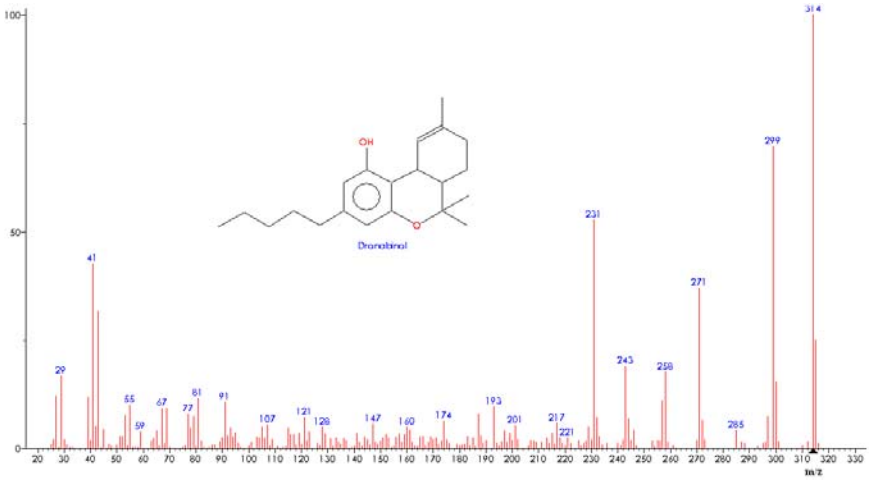
As it can be observed, Δ^9–THC, CBN and CBG are present in all cannabis plants and there is an insignificant difference between the values of retention time of the same component, whatever type of cannabis plant. Other components such as CBD, Δ^8–THC, THCV and CBC that appear as missing in some types of cannabis, especially CBD, are actually present in much smaller concentration than the first ones. However, the obtained results suggest that both sample preparation method and analytical protocol used are appropriate for this type of analysis.



CBD



CBN



Δ^9–THC

Fig. 2. Mass spectra of major cannabinoids in herbal cannabis

Phenotype assessment by macroscopic examination

The macroscopic examination revealed that all the herbal cannabis are female and have characteristics in common with some types of cannabis cataloged as drug type. For example, the herbal cannabis having denser and not too leafy buds could be likened to so call AK 47 cannabis strain while the herbal cannabis having buts with a sweet smell and that are coated with light tan crystals could be likened to so call White Russian. The herbal cannabis having smaller and thin buds is originated from Romania. The differences between the six types of herbal cannabis could be observed from the photos presented in Fig. 3. The variation in physical characteristics could be attributed to the seed strain as well as to the environmental factors such as light, water, nutrients and space where the cannabis plants have developed.

Cannabis AK 47

Cannabis Moby Dick

Cannabis White Russian

Cannabis AK Moon

Cannabis Ice Bull

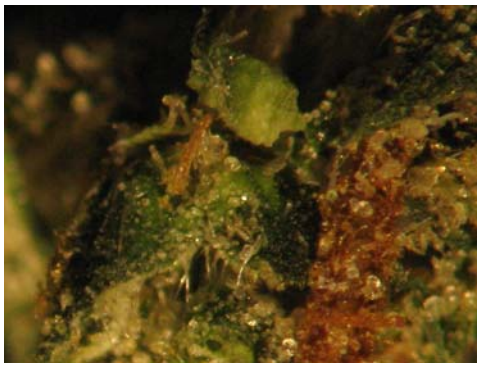
Cannabis Ro

Fig. 3. Types of herbal cannabis subjected to experimental investigation in this work

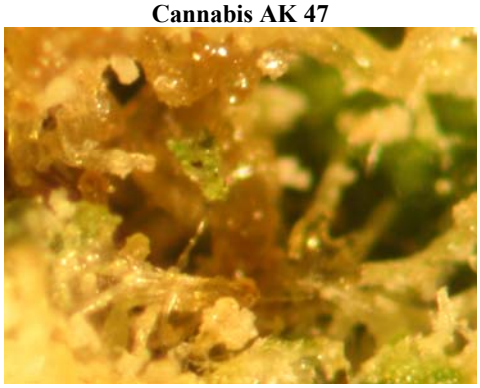
Phenotype assessment by microscopic examination

The phenotype of herbal cannabis can be assessed based on the type of microscopic structures on the surface of the plant called trichomes. As the Fig. 4 shows, all the six herbal cannabis subjected to experimental investigation in this work have a series of glandular trichoms where the cannabis resin is produced and stored. These are of the sessile glands type, small bulbous glandular trichoms and multicellular stalked glandular trichoms. The density of these trichoms on the flower structures of the female cannabis plants decreases from cannabis AK 47 to cannabis Ro in the following order: cannabis AK 47, cannabis Moby Dick, cannabis White Russian, cannabis AK Moon, cannabis Ice Bull and cannabis Ro. It is also expected that the content of psychoactive component of the herbal cannabis, namely Δ^9-THC, to decrease in the same order. However, the presence of glandular trichoms in a relatively high abundance on the each type of herbal cannabis suggests that all of these are drug type.

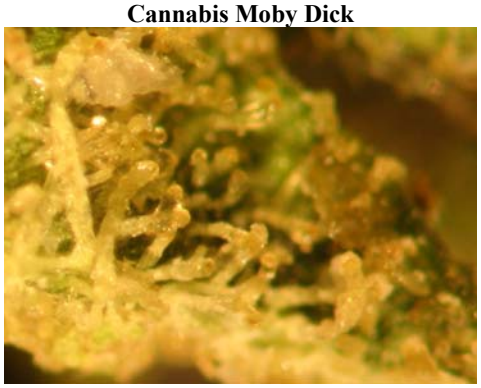
Cannabis AK 47



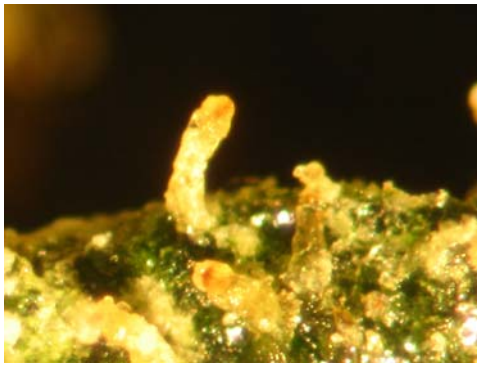
Cannabis Moby Dick



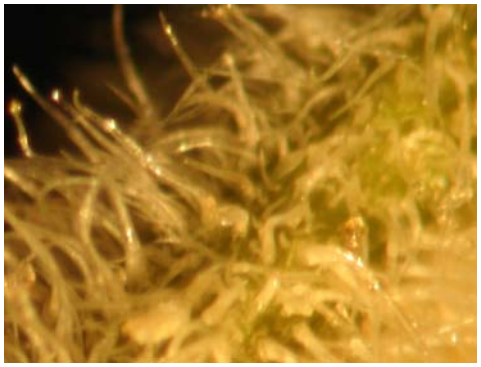
Cannabis White Russian



Cannabis AK Moon



Cannabis Ice Bull



Cannabis Ro

Fig. 4. Microscopic imagines of the six herbal cannabis types subjected to experimental investigation in this work

Chemical phenotype assessment

Based on GC-FID analysis, the concentration of major cannabinoids in the herbal cannabis was determined. The results are presented in Table 2. For example, gas chromatograms only for cannabis AK 47, cannabis White Russian and cannabis Ro are presented in Fig. 5. From this table, it can be seen that all the six samples have a high content in Δ^9–THC, higher than UE limit of 0.3%.

According to the UE guideline [10] these herbal cannabis can be classified as drug-type.

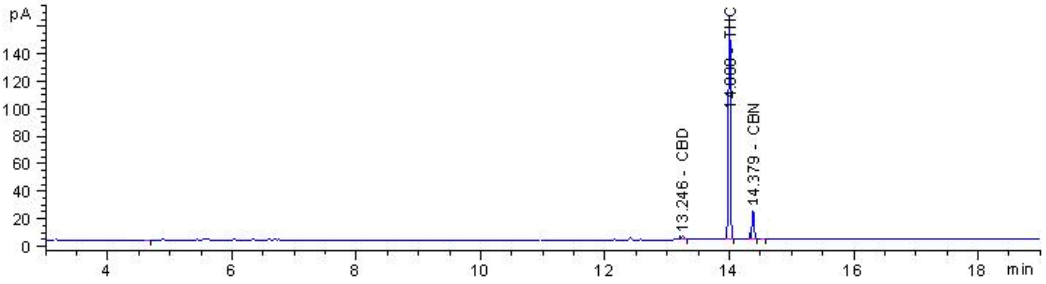
Table 2

Concentration of the major cannabinoids in herbal cannabis

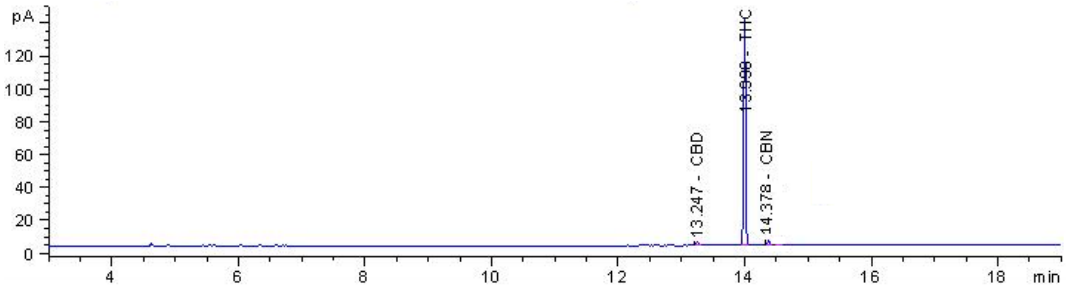
Cannabinoid, %	Cannabis type*					
	AK 47	***Moby Dick***	***White Russian***	***AK Moon***	***Ice Bull***	***Ro***
Δ^9-THC	23.11	22.03	19.13	19.09	17.59	1.87
CBN	3.07	1.91	0.76	0.42	0.34	0.45
CBD	0.41	0.35	0.29	0.33	0.32	4.25

*were marked from AK 47 to Ro in order of increasing concentration in Δ^9-THC

Based on both Δ^9-THC/CBD ratio and Phenotypic Index two chemotypes of herbal cannabis were identified. The results are presented in Table 3. Chemotype I or drug–type was identified for cannabis AK 47, cannabis Moby Dick, cannabis White Russian, cannabis AK Moon and cannabis Ice Bull, while chemotype III or fiber–type was identified for cannabis Ro. Moreover, as it is shown in the Fig. 6, when the Δ^9–THC content is plotted against CBD content and a limit of 0.5 % of both Δ^9–THC and CBD is established as a boundary value between the tree chemotypes, it appears that cannabis originated from Romania is of intermediary–type. However, even if there is a conflicting classification of chemotype for herbal cannabis originated from Romania as a function of the phenotypic indicator used, it can be concluded that all the herbal cannabis subjected to experimental investigation in this work are drug–type.



Cannabis AK 47



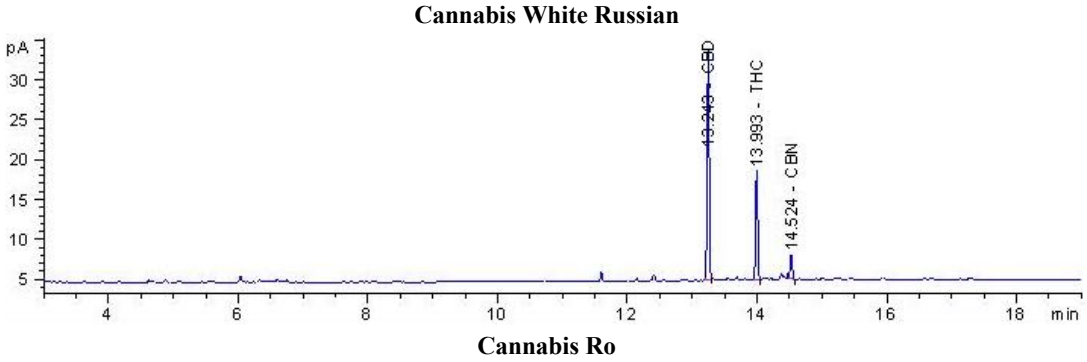


Fig. 5. Gas chromatograms of herbal cannabis

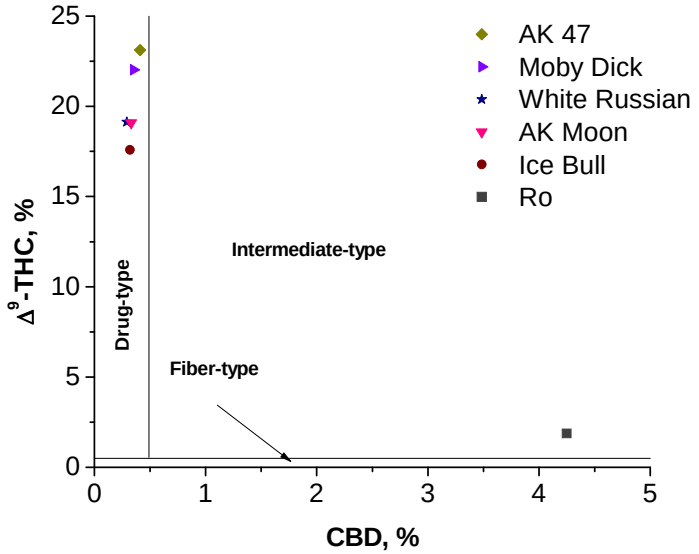


Fig. 6. Δ^9-THC *vs.* CBD content of the six types of herbal cannabis

Table 3

Chemical phenotype of the herbal cannabis

Cannabinoid, %	Cannabis type					
	AK 47	***Moby Dick***	***White Russian***	***AK Moon***	***Ice Bull***	***Ro***
Δ^9-THC	23.11	22.03	19.13	19.09	17.59	1.87
Δ^9-THC/CBD	56.37	62.94	65.97	57.85	54.97	0.44
Phenotypic Index	63.85	68.40	68.59	59.12	56.03	0.55
Chemotype	I	I	I	I	I	I

Higher chemical potency of these cannabis plants suggests that these were grown under controlled environmental conditions. Moreover, as these specific cannabis strains resulted from many trials of breading, it is likely that these plants have been propagated from clones (successful "mother" plants) and grown indoors.

Estimation of the age of cannabis samples

The major degradation product of Δ^9–THC, namely CBN, does not exist in freshly herbal cannabis. If CBN is present then Δ^9–THC has started to degrade and the herbal cannabis begins to lose its potency. Based on the work of Ross and Elsohly [10] and on the experimental data obtained in this work from GC–FID analysis, the age of all types of herbal cannabis was estimated. The results are presented in Table 4.

Table 4

Age of the herbal cannabis

Cannabinoid, %	**Cannabis type**					
	AK 47	***Moby Dick***	***White Russian***	***AK Moon***	***Ice Bull***	***Ro***
Δ^9-THC	23.11	22.03	19.13	19.09	17.59	1.87
CBN	3.07	1.91	0.76	0.42	0.34	0.45
CBN/Δ^9-THC	0.13	0.09	0.04	0.02	0.02	0.24
Age, years	0.18	0.17	0.15	0.15	0.15	0.21

As it can be seen from Table 4, all the cannabis plants have an age up to two and a half months. Thus, it may be concluded that all the herbal cannabis have the same degree of freshness and it is feasible to assess and then compare their phenotype between them.

4. Conclusions

Based on both macroscopic and microscopic examination, it results in the cannabis plants subjected to experimental investigations in this work are similar to a series of hybrid cannabis strains such as cannabis AK 47, cannabis Moby Dick, cannabis White Russian, cannabis AK Moon, cannabis Ice Bull. Only one was identified to be originated from Romania.

The chemical characterization of the cannabis plants subjected to experimental investigations in this work revealed that these contain a high content of Δ^9–THC and subsequently a high potency. Higher potency of these cannabis plants suggests that these were propagated from clones and grown indoors. Finally, it could be concluded that these hybrid varieties of herbal cannabis are drug-type.

The experimental results concerning age of the above cannabis plants revealed that all of these have the same degree of freshness and it is feasible to assess and then compare their phenotype between them.

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