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# An approach to the evaluation of the potency of cannabis resin in Madrid: A health hazard?

## *Aproximación a la evaluación de la potencia de la resina de cannabis en Madrid: ¿Un riesgo para la salud?*

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## Abstract

The present study investigates the concentration of Delta (9)-tetrahidrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in 60 samples of cannabis resin acquired on the streets of Madrid region and its potential danger to consumers' health. Additionally, we study the possible correlation between the potency of samples and their organoleptic characteristics. The analysis of cannabinoids was carried out using a high performance liquid chromatography (RP-HPLC-UV). To classify samples, a strength scale based on THC content was established. THC content in 76.7% of the samples was higher than 15%. This potency allows these samples to be classified as Schedule I or drugs with "unacceptable risk" for human health. THC content in 36.7% of the samples was 28.8% on average, which means very high potency. The mean CBD content was 5%, while the correlation between the CBD/THC ratio and potency was negative. The mean content of CBN was 1.74% and the CBN/THC ratio also showed a negative correlation in respect to potency. When investigating the possible correlation between sample potency and organoleptic characteristics, those samples which simultaneously presented sticky texture, high elasticity and light brown colour had very high potency, with an average THC content of 28.7%. Our study shows that the THC content of most of the cannabis that can be purchased in Madrid region is over 15% and poses a health hazard. Additionally, we demonstrate for the first time that only those samples with very high potency can be directly associated with certain organoleptic characteristics.

***Keywords:*** cannabis resin, cannabis potency, THC, CBD, CBN

## Resumen

El presente estudio investiga la concentración de Delta(9)-tetrahidrocannabinol (THC), cannabidiol (CBD) y cannabinol (CBN) en 60 muestras de resina de cannabis adquiridas en las calles de Madrid y su potencial riesgo para la salud del consumidor. Adicionalmente, estudiamos la posible asociación entre la potencia de las muestras y sus características organolépticas. El análisis de cannabinoides se llevó a cabo mediante cromatografía líquida de alta resolución (RP-HPLC-UV). Atendiendo al contenido en THC se estableció una escala de potencia para clasificar las muestras. El 76,7% de las muestras tenía un contenido en THC superior al 15%, esta potencia las cataloga como drogas de Grado I con "riesgo inaceptable" para la salud. El 36,7% de las muestras presentaron un contenido medio en THC del 28,8% (potencia muy alta). El contenido medio en CBD fue del 5% y el de CBN 1,74%; ambas ratios, CBD/THC y CBN/THC, mostraron una correlación negativa con la potencia. Al investigar la posible asociación entra potencia y características organolépticas, se observó que las muestras que presentaban a la vez una textura pegajosa, una elasticidad alta y un color marrón claro, tenían una potencia muy alta, con un contenido medio en THC del 28,7%. Nuestro estudio muestra que el contenido en THC de la mayoría de la resina de cannabis que puede adquirirse en Madrid es superior al 15% y supone un elevado riesgo para la salud. Adicionalmente, demostramos por primera vez que solo aquellas muestras con una potencia muy alta pueden asociarse directamente con ciertas características organolépticas.

***Palabras clave:*** resina de cannabis, potencia del cannabis, THC, CBD, CBN

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The increase in adverse effects caused by cannabis use has generated growing concern in recent years. Although illegal in most countries, this drug is the most widely used drug of abuse in the world (United Nations Office on Drugs and Crime [UNODC], 2018). Indeed, in Spain, cannabis is the illicit substance which causes the highest number of first admissions for treatment in outpatient centres, representing 40.7% of these, according to statistics on admissions for psychoactive substance use (Delegación del Gobierno de España para el Plan Nacional sobre Drogas, 2019). Likewise, ER statistics on psychoactive substance use show that cannabis is linked to over 40.1% of such emergency cases (Delegación del Gobierno de España para el Plan Nacional sobre Drogas, 2019).

In 2017, the prevalence of cannabis use among young adults (aged 15 to 34 years) in Spain was 18.3%, with a prevalence among students aged 15-16 years of 27%, a value much higher than the European average of 16% for this age group (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2019). Among the 35 countries included in this study, Cyprus and Greece can be highlighted as having the lowest prevalences among 15- to 16-year-old students (7% and 9%, respectively), with 15% in Portugal, and the highest prevalences in Italy (27%) and France, at 31%.

Cannabis contains more than 500 different compounds, of which approximately 100 are cannabinoids. Of the latter, the most relevant, given both their great presence and the effects they produce on the individual, are Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN); for this reason, they are also the compounds whose content is usually analysed in most studies (Dujourdy & Besacier, 2017; Niesink, Rigter, Koeter & Brunt, 2015; Pijlman, Rigter, Hoek, Goldschmidt & Niesink, 2005; Potter, Hammond, Tuffnell, Walker & Di Forti, 2018; Zamengo, Frison, Bettin & Sciarrone, 2014).

The active components of cannabis mimic the effects of endogenous cannabinoids, activating specific cannabinoid receptors, in particular CB1, which is predominantly found in the central nervous system (Bridgeman & Abazia, 2017; Matsuda, Lolait, Brownstein, Young & Bonner, 1990), and CB2, located mainly in cells related to immune function (Abrams & Guzman, 2015). Both are protein-based membrane receptor and associated with the G protein (Matsuda et al., 1990; Ramos Atance & Fernandez Ruiz, 2000).

Being the most important active ingredient responsible for the psychoactive properties of the plant (Casajuana Köguel, López-Pelayo, Balcells-Olivero, Colom & Gual, 2018; Gaoni & Mechoulam, 1964; Waller, 1971), THC is also the main reason behind the recreational use of cannabis derivatives. The content of this substance is expressed as a percentage of product weight and determines the potency of cannabis (Niesink & van Laar, 2013). High THC content can increase anxiety, depression and psychotic symptoms (Di Forti et al., 2009; Hall & Degenhard, 2009). Likewise, recent research indicates that the occasional use of cannabis with high THC content impairs cognitive capacity, in particular memory and emotional processing (Colizzi & Bhattacharyya, 2017).

However, the effects of cannabis on humans depend not only on THC content, but also on the amount of other substances and the ratio between them. One of these compounds is CBD, with the CBD/THC ratio being very important (Casajuana et al., 2018; Colizzi et al., 2017; Lafaye, Karila, Blecha & Benyamina, 2017; Niesink et al., 2013). Previous studies have shown that there is a direct relationship between increasing potency (THC) and decreasing CBD concentration (ElSohly et al., 2016; Potter et al., 2018). Logically, this circumstance can have serious implications on the health of consumers. Although CBD lacks psychoactive effects, it is the second most important active ingredient in the composition of cannabis. It provides a sedative, relaxing, antiemetic and analgesic effect, which favours its therapeutic use (Lleonart, 2018). CBD is believed to counteract some of the harmful effects of THC, at least in part (Niesink et al., 2013), as it promotes relaxation and possibly even provides some antipsychotic effect. Some evidence, still limited, suggests that the administration of CBD can improve cognitive aspects in cannabis users, although not in individuals with neuropsychiatric disorders (Colizzi et al., 2017). Consequently, lower CBD concentrations reduce the protective effect generated by this active ingredient, in contrast to the damage induced by THC in emotional processing and memory (Colizzi et al., 2017).

For its part, CBN is a compound derived from the oxidation of THC, as a result of the passage of time or inadequate storage conditions. Since CBN is not present in the fresh product (Ross & ElSohly, 1997), it is considered a primary product of THC degradation, with a psychoactive effect up to ten times lower. These characteristics mean that the relationship between CBN and THC concentrations (the CBN/THC ratio) is used as an indicator of the freshness of the cannabis samples or their “age” (Ross et al., 1997).

In Spain, cannabis resin (or hashish) is an illegal drug of abuse which can be acquired mainly through small-scale street sales. This type of product is clearly not subject to any quality control, nor is its potency monitored routinely. It must also be taken into account that hashish consumers consider some of external characteristics, such as texture, elasticity and colour, as quality indicators in identifying products with greater potency (i.e. with higher THC content). Thus, in the opinion of users, brown samples, with great elasticity and a sticky texture, would theoretically be of the best quality.

The street sale of cannabis in the Madrid region has been the subject of a previous study, which analysed the degree of adulteration and contamination of samples (Pérez-Moreno, Pérez-Lloret, González-Soriano & Santos-Álvarez, 2019). However, neither the potency of this type of sample nor the possible relationship between potency and some of the organoleptic characteristics valued by consumers, such as texture, elasticity and colour, was determined. This study therefore extends our previous work, investigating the concentration of the three main active ingredients, that is, THC, CBD and CBN of the cannabis resin sold on the streets in the Madrid area and its potential danger to the health of users. We also study the possible association between the potency of the samples and their organoleptic characteristics.

## Materials and methods

### Samples

A total of 60 samples of cannabis resin acquired on the street were analysed. The sampling, at a rate of 5 samples per month over the 12 months of the year, was designed on the one hand to minimize possible seasonal variation and, on the other, to try to guarantee that samples came from different consignments. The samples were collected in different localities of the Community of Madrid (Madrid capital (n = 17), northern zone of the region (n = 10), southern zone (n = 15), eastern zone (n = 10) and western zone (n = 8)) to achieve as representative a population as possible. Although the number of samples may appear somewhat small, it can serve as a first approximation in the study; the difficulty of obtaining samples whose sale is not legal should also be taken into account. After purchase, the samples were stored in sterile bags and stored frozen at -40ºC until subsequent analysis, carried out in a period not exceeding 15 days after acquisition. The first step consisted in assessing the organoleptic characteristics of interest in this study in each case, that is, colour, elasticity and texture, and subsequently to analyse cannabinoid content: Delta (9) -tetrahydrocannabinol (THC), cannabidiol (CBD ) and cannabinol (CBN). Content was expressed as a percentage of cannabis resin weight. The mean weight of the samples was 6.9 g (range: 4.9 - 9.9 g). Prices ranged from 20 to 40 euros. This is not a minor detail since it means that the purchase of cannabis in the Community of Madrid can be accessible to practically any group.

### Organoleptic characteristics

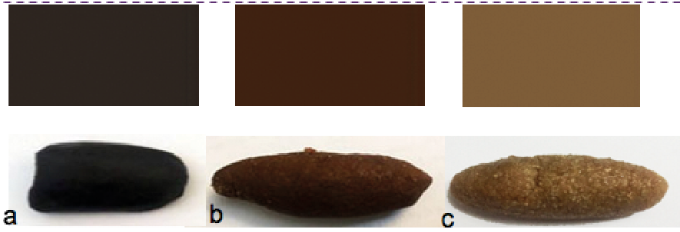
To define colour, three shades of brown were used: dark, medium and light. To minimize the degree of subjectivity in colour appreciation as far as possible, each sample was compared to the colour scale shown in Figure 1. Elasticity was also classified into three levels: zero, medium or high. Zero elasticity describes when the sample broke with minimum traction; average if, when bending the sample, it was possible to join both ends but then fractured when returning to its initial position; and high if, after bending it and joining both ends, it was possible to return it to the initial position without breaking. Texture was considered sticky if, after slight pressure, the sample adhered to the glove fingers or dry if the sample did not adhere at all.

### Cannabinoid analysis by RP-HPLC-UV

The active components of the samples (THC, CBN, CBD), as well as their acidic or inactive forms (THC-A and CBD-A), were analysed according to the method recommended by the American Herbal Pharmacopeia (ElSohly & Chandra, 2013). The method consists of three steps: 1) extraction of the compounds with methanol, 2) quality control of the method, and 3) identification and quantification of the compounds using chromatographic techniques.

1. To extract the compounds, 5 ml of methanol (99% LC methanol, Sigma-Aldrich) was added to 50 mg from each sample, previously ground with an electric grinder for 20 seconds at 3500 rpm. This mixture was sonicated in an ultrasound bath for 15 minutes before sieving with a 0.45 µm pore diameter filter. The extract was analysed by chromatography at this initial concentration and at the following dilutions: a) 200 µl

**Figure 1**
*Colour scale used to classify cannabis resin samples: a) dark brown; b) medium brown; c) light brown*



of the initial solution diluted to 1 ml with methanol; and b) 16 µl of the starting solution diluted to 1 ml with methanol.

The standard samples of active ingredients, or reference standard cannabinoids (purity = 99%, Lipomed AG) for calibration were: Δ9-THC (1 mg/ml in ethanol and 50 mg/ml in ethanol), CBN, CBD, THC-A and CBD-A (1 mg/ml in ethanol).

2. For quality control of the method, several parameters were used: to check the linearity of the chromatographic response (r2 ≥ 0.99), the correlation coefficient of each extract and its dilutions were assessed; limit of quantification (LOQ) for the compounds was 0.1 µg/ml; daily relative standard deviation (intra- and inter-day) (% RSD) for retention times and areas under peaks was ≤ 5%; finally, sample recovery was ≥ 95%.
3. Cannabinoid analysis by RP-HPLC-UV: analysis of THC, CBD and CBN was carried out using a high performance liquid chromatography system with a column oven (Jasco Co-2060 Plus Intelligent Column Thermostat), a quaternary low pressure gradient pump (Jasco pump PU 2089 Plus Intelligent) and an automatic sample injection system (Jasco AS-2057 Plus Intelligent Sampler), coupled with an ultraviolet-visible detector (Jasco UV-2075 Plus Intelligent UV/Vis Detector). The chromatographic separation of the compounds was carried out using a C18 Agilent column (150 x 46 mm, 5 µm). For each sample, 10 µl of the extract at the initial concentration, as well as of the two dilutions, were injected into the system. Throughout the test, elution from the column was achieved by a linear elution gradient (total time 20 minutes) using a mixture of water (LC grade, Panreac) and acetonitil (LC grade, Sigma-Aldrich) in a 90% proportion: 10% to a final mixture in the ratio 10%: 90%. The column oven temperature was kept at 35°C.

The total concentration of THC and CBD was the sum of their acidic forms plus their decarboxylated forms. CBN, as a product obtained by the degradation of THC, was generated in a neutral fashion (Fig. 2).

Assessment of sample potency

The THC richness scale published by the Instituto Nacional de Toxicología y Ciencias Forenses [INTCF] (2013) was used to assess the potency of the samples. The cannabis resin was classified as: very high potency (THC > 25%); high potency (20% < THC ≤ 25%); medium potency (15% < THC ≤ 20%); low potency (10% < THC ≤ 15%); and very low potency (5% < THC ≤ 10%).

## Statistical analysis

All data were registered in a database using the Excel program. Statistical analysis was performed with the SAS 9.4 program.

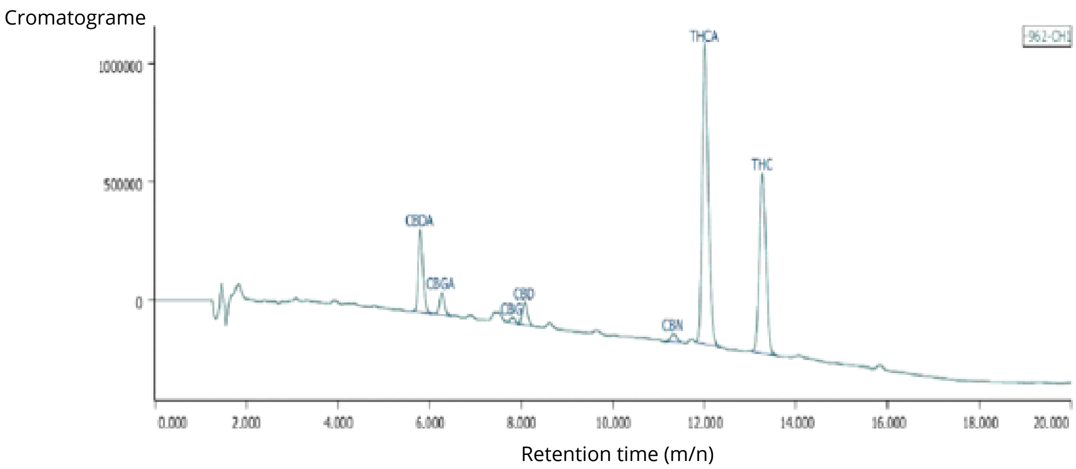
Qualitative variables (colour, elasticity and texture) are shown in distribution tables with frequencies and percentages. For quantitative variables (concentration of THC, CBD and CBN), the number of cases (n), the mean, the standard deviation (SD) and the minimum and maximum values were recorded.

Qualitative variables were compared using Pearson's chi square (comparison of percentages) or Fisher's exact test (when frequencies were too low for any category).

The relationship between dichotomous qualitative variables (texture: dry or sticky) and ordinal variables (e.g., elasticity: zero = 0, medium = 1, high = 2; potency scale) was assessed using the Wilcoxon rank sum test.

The relationship between qualitative variables with more than two levels (e.g.: colour) and ordinal variables was assessed using the non-parametric Kruskal-Wallis test. If significant, a nonparametric multiple comparisons test was used.

**Figure 2**
*Chromatogram of a real sample analysed*



Given the significant lack of fit to the normal distribution produced by the numerical variables studied, the relationship between qualitative and numerical variables was assessed with the same tests as for the ordinal ones (Wilcoxon and Kruskal-Wallis).

Finally, to determine the relationship between quantitative and ordinal variables (e.g.: power scale), the Spearman rank correlation coefficient was calculated.

A 95% confidence level was established in all analyses, i.e., statistical significance was found at $p < 0.05$.

## Results

The distribution of the cannabis resin samples on the potency scale, as well as their average THC, CBD and CBN content and respective CBD/THC and CBN/THC ratios, are shown in Table 1. As can be seen, the average THC content of 76.7% of the samples was higher than 15%, distributed as follows: 36.7% of the samples had very high potency (n = 22), 20% (n = 12) were potent, while another 20% (n = 12) were considered of medium strength. Regarding CBD content, it is interesting to note that those samples with very high potency were also those that had the most CBD, up to an average of 6.1%; conversely, those samples with very low potency were those containing the least CBD, with an average value of 3.1%. However, the CBD/THC ratio had a negative correlation with respect to potency. Regarding CBN content, the most potent samples were those with a lower average value, specifically 0.68%, while the highest amount, up to 4%, was directly related to the least potent. The CBN/THC ratio, which refers to the freshness of the sample, was also negatively correlated with respect to sample strength, with a range from 0.024 (in very high potency samples) to 0.490 (in very low potency samples) (Table 1).

Table 2 relates sample strength with each of the organoleptic characteristics of interest in this study: texture, elasticity and colour. Most of the samples with a sticky texture (56.7%), high elasticity (94.4%) and light brown colour (81.2%) were found to be highly potent. Furthermore, no samples with high elasticity and light brown colour had very low potency; in the case of texture, we did find a sample with a sticky texture and very low potency. However, in Table 2 it is also striking that a high percentage of samples with dry texture, zero or medium elasticity and dark or medium brown colour presented high or medium potency. On the other hand, when analysing the average THC content of the samples based on their organoleptic characteristics (Table 3), it was observed that the samples with the three characteristics, sticky texture, high elasticity and light brown colour, had a medium THC content of 23.1%, 28.1% and 27%, respectively, higher than that detected in the rest of the samples with other characteristics. As can be seen, the average CBD content of the samples with these three characteristics was also higher than that found in the rest of the samples with other organoleptic characteristics (except for texture). Another interesting fact is that in the CBD/THC ratio, when the three organoleptic characteristics of interest are considered, no statistically significant differences are observed. However, when the CBN/THC ratio is calculated, only in the case of texture does the value turn out to be not significant.

Those samples that simultaneously met the three characteristics (sticky texture, high elasticity and light brown colour, n = 11) had higher THC content (28.7%) and lower CBN content (0.89%) than the rest of samples (n = 49; THC 19.2% and CBN 1.93%), differences which were statistically significant (Table 4).

**Table 1**
*Average content (%) of THC, CBD, CBN, and CBD/THC and CBN/THC ratios of the cannabis resin samples as a function of the potency scale*

| Potency nº (%) | THC (%) | | CBD (%) | | CBN (%) | | ratio CBD/ THC | | ratio CBN/ THC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± DE | Range | Mean ± DE | Range | Mean ± DE | Range | Mean ± DE | Range | Mean ± DE | Range |
| Very high 22 (36.7) | 28.8 ± 2.2 | 25.3 – 33.0 | 6.1 ± 1.0 | 4.2 – 8.8 | 0.68 ± 0.6 | 0.2 – 3.2 | 0.21 ± 0.0 | 0.2 – 0.3 | 0.024 ± 0.02 | 0.006 – 0.107 |
| High 12 (20) | 22.8 ± 1.9 | 20.0 – 25.0 | 5.2 ± 1.6 | 3.0 – 8.0 | 1.51 ± 0.7 | 0.2 – 2.3 | 0.23 ± 0.1 | 0.2 – 0.4 | 0.069 ± 0.04 | 0.008 – 0.115 |
| Medium 12 (20) | 18.3 ± 1.1 | 15.0 – 19.2 | 4.6 ± 1.3 | 3.0 – 7.0 | 2.32 ±1.0 | 0.7 – 3.8 | 0.25 ± 0.1 | 0.2 – 0.4 | 0.129 ± 0.06 | 0.037 – 0.211 |
| Low 10 (16.7) | 11.7 ± 1.0 | 10.2 – 14.0 | 3.7 ± 0.9 | 2.3 – 5.0 | 2.73 ± 0.8 | 1.8 – 4.3 | 0.32 ± 0.1 | 0.2 – 0.5 | 0.235 ± 0.07 | 0.167 – 0.391 |
| Very low 4 (6.7) | 8.4 ±1.4 | 6.6 – 9.5 | 3.1 ± 1.4 | 1.7 – 4.6 | .00 ± 0.6 | 3.5 – 4.5 | 0.36 ± 0.2 | 0.2 – 0.5 | 0.490 ± 0.14 | 0.368 – 0.682 |
| Average | 21.3 ± 7.3 | 6.6 – 33.0 | 5.0 ± 1.5 | 1.7 – 8.8 | 1.74 ± 1.2 | 0.2 – 4.5 | 0.25 ± 0.1 | 0.2 – 0.5 | 0.120 ± 0.14 | 0.006 – 0.682 |

*Nota.* THC: Δ9-tetrahydrocannabinol; CBD: cannabidiol; CBN: cannabinol. Potency scale: very high potency: THC > 25%; high potency: 20%> THC ≤ 25%; medium potency: 15%> THC ≤ 20%; low potency 10% > THC ≤ 15%; very low potency: 5% > THC ≤ 10%.

## Discussion

In this study, we analysed the potency of cannabis resin available on the streets in the Madrid region. The collection of cannabis resin samples was spread across a whole year and across all areas of the region to ensure that the samples did not come from the same consignment or the same vendor. We consider that this sampling can provide a fairly good idea of the potency of cannabis resin consumed in the Madrid region. It should be noted that this is the first study of its kind carried out in the Autonomous Community of Madrid.

To this end, the quality scale published by the INTCF (2013) was used as a yardstick. However, rather than the term "THC concentration", for this study the term

**Table 2**
Distribution of samples (number and percentage) of cannabis resin on the potency scale according to their organoleptic characteristics

| Organoleptic characteristics (n) | Potency n (%) | | | | | P value |
|---|---|---|---|---|---|---|
| | very high | high | medium | low | very low | |
| Texture | | | | | | |
| dry (30) | 5 (16.7) | 9 (30) | 10 (33.3) | 3 (10) | 3 (10) | <0.05 |
| sticky (30) | 17 (56.7) | 3 (10) | 2 (6.7) | 7 (23.3) | 1 (3.3) | |
| Elasticity | | | | | | |
| zero (22) | 2 (9.1) | 6 (27.3) | 8 (36.4) | 3 (13.6) | 3 (13.6) | <0.0001 |
| medium (20) | 3 (15) | 6 (30) | 4 (20) | 6 (30) | 1 (5) | |
| high (18) | 17 (94.4) | 0 | 0 | 1 (5.6) | 0 | |
| Colour | | | | | | |
| dark brown (26) | 4 (15.4) | 6 (23.1) | 6 (23.1) | 6 (23.1) | 4 (15.4) | <0.0005 |
| medium brown (18) | 5 (27.8) | 5 (27.8) | 4 (22.2) | 4 (22.2) | 0 | |
| light brown (16) | 13 (81.2) | 1 (6.2) | 2 (12.5) | 0 | 0 | |

*Note.* THC: Δ9-tetrahydrocannabinol. Very high potency: THC > 25%; high potency: 20% > THC ≤ 25%; medium potency: 15% > THC ≤ 20%; low potency 10% > THC ≤ 15%; very low potency: 5% > THC ≤ 10%.

**Table 3**
*Comparison of the mean content (%) of THC, CBD, CBN and ratios CBD/THC and CBN/THC of the cannabis resin samples according to their organoleptic characteristics*

| Characteristics organoleptic (n) | THC (%) | | CBD (%) | | CBN (%) | | ratio CBD/ THC | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± DE | p | Mean ± DE | p | Mean ± DE | p | Mean ± DE | p | Mean ± DE | p |
| Texture | | | | | | | | | | |
| dry (30)<br>sticky (30) | 19.5 ± 6.1<br>23.1 ± 8.0 | <0.05 | 4.80 ± 1.46<br>5.29 ± 1.62 | NS | 1.84 ± 1.14<br>1.64 ± 1.34 | NS | 0.26 ± 0.09<br>0.25 ± 0.08 | NS | 0.13 ± 0.14<br>0.11 ± 0.13 | NS |
| Elasticity | | | | | | | | | | |
| null (22)<br>medium (20)<br>high (18) | 18.1 ± 6.2<br>18.6 ± 6.2<br>28.1 ± 4.8 | <0.0001a<br><0.0001b | 4.42 ± 1.31<br>4.84 ± 1.67<br>6.02 ± 1.22 | <0.005[a]<br><0.05[b] | 2.26 ± 1.15<br>1.93 ± 1.24<br>0.89 ± 0.90 | <0.0005a<br><0.01b | 0.26 ± 0.07<br>0.28 ± 0.11<br>0.22 ± 0.05 | NS | 0.17 ± 0.16<br>0.14 ± 0.13<br>0.04 ± 0.07 | <0.0005a<br><0.005b |
| Colour | | | | | | | | | | |
| dark brown (26)<br>medium brown (18)<br>light brown(16) | 18.0 ± 7.5<br>20.9 ± 6.5<br>27.0 ±3.9 | <0.0005c<br><0.01d | 4.26 ± 1.30<br>5.47 ± 1.54<br>5.83 ± 1.41 | <0.005[c] | 2.42 ± 1.30<br>1.53 ± 0.93<br>0.86 ± 0.75 | <0.0001c | 0.26 ± 0.09<br>0.28 ± 0.09<br>0.22 ± 0.05 | NS | 0.19 ± 0.17<br>0.09 ± 0.07<br>0.03 ± 0.03 | <0.00005c |

*Note.* THC: Δ9-tetrahydrocannabinol; CBD: cannabidiol; CBN: cannabinol. a: High elasticity versus zero elasticity; b: high elasticity versus medium elasticity; c: dark brown colour versus light brown colour; d: medium brown colour versus light brown colour.

**Table 4**
*Comparison of the average content (%) of THC, CBD, CBN and ratios CBD/THC and CBN/THC between the groups of cannabis resin samples*

| Groups nº (%) | THC (%) | | CBD (%) | | CBN (%) | | CBD/THC ratio | | CBN/THC ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± DE | ratio | Mean ± DE | ratio | Mean ± DE | ratio | Mean ± DE | ratio | Mean ± DE | ratio |
| Group 1<br>11 (18.3) | 28.7 ± 2.2[a] | 25.5 – 33.0 | 6.0 ± 1.1[b] | 4.2 – 8.0 | 0.89 ± 0.86b | 0.30 – 3.20 | 0.210 ± 0.047 | 0.150 – 0.308 | 0.031 ± 0.029[c] | 0.009 – 0.107 |
| Group 2<br>49 (81.7) | 19.6 ± 7.0 | 6.6 – 33.0 | 4.8 ± 1.6 | 1.7 – 8.8 | 1.93 ± 1.24 | 0.20 – 4.50 | 0.262 ± 0.088 | 0.150 – 0.500 | 0.140 ± 0.141 | 0.006 – 0.682 |

*Note.* Group 1: samples with simultaneously sticky texture, high elasticity and light brown colour. Group 2: other samples. THC: Δ9-tetrahydrocannabinol; CBD: cannabidiol; CBN: cannabinol. a: p < 0.0005; b: p < 0.05; c: p < 0.005.

"potency" was preferred, which in our opinion better expresses the strength, despite the negative connotations in this case, of a sample high in THC. The samples were classified according to their content of this compound as having very high, high, medium, low and very low potency.

When analysing the concentrations of THC, CBD and CBN, it is very important to take into account the type of cannabis derivative under analysis (marijuana, seedless, cannabis resin, imported cannabis, national cultivation, etc.) (INTCF, 2013; Niesink et al. al., 2015; Pijlman et al., 2005; Potter et al., 2018; Zamengo et al., 2014). Given the increase in the THC content of cannabis and the greater knowledge of the risks it poses for the health of users, especially young people, it has been suggested that the forms of potent cannabis (containing over 15% THC) be reclassified as Grade I drugs, that is, drugs "with unacceptable risk" to health, thereby equating them to heroin and cocaine (Niesink et al., 2015; Van Laar, Nan der Pol & Niesink, 2016). Our study shows that the cannabis resin (or hashish) acquired on the streets of Madrid has high average THC content. It should be noted that this is not an isolated value, but rather reflects very similar figures found in previous studies carried out in other regions of Spain (Asociación Vasca de Personas Usuarias de Drogas para la Reducción de Riesgos [Ai Laket], 2016; Energy Control, 2016) or in France (Dujourdy et al., 2017), which support our results. One of our study's findings of particular relevance is that three quarters of the samples contained over 15% THC, which would put them within Group I, i.e. drugs with an "unacceptable risk" to health. Furthermore, over a third of these samples were very potent (with a THC content above 25%). These data are much higher than those found in other European countries such as Italy and the Netherlands, where the average THC content of the cannabis resin samples are reported to be 8.9% and 16.5%, respectively (Niesink et al., 2015; Zamengo et al., 2014). In other words, the cannabis that can be purchased and consumed in the Madrid area would be more harmful to consumers than that available for purchase in other countries of the European Community. However, in recent years there has been a general increase in the potency of cannabis samples (Dujourdy et al., 2017; ElSohly et al., 2016; Potter et al., 2018), and the different years in which the various studies were carried out could thus explain, at least in part, the higher THC content of the cannabis resin samples analysed in this study compared to that found in other European countries.

The adverse psychological effects induced by THC can be partially offset by the CBD content of cannabis samples (Casajuana et al., 2018; Colizzi et al., 2017; Lafaye et al., 2017; Niesink et al. 2013). When estimating the risk of psychotic effects from cannabis, it is therefore important to determine the CBD/THC ratio. It might be thought that cannabis resins with a CBD/THC ratio close to unity would be less harmful to the consumer than those with lower values. Our results indicate that the more potent a sample is, the lower its CBD/THC ratio and even samples with medium potency have a ratio far below unity. These data, added to the high THC content of the samples, suggest that the cannabis available in different parts of the Madrid area would have a very significant negative impact on the health of the regular user. We have not found studies that analyze the THC, CBD and CBN content of the cannabis resin consumed in the Madrid region, but there have been earlier studies in Spain (Ai Laket, 2016; Energy Control, 2016) that collected some similar values for hashish sold in other autonomous communities, which would confirm the high potency of cannabis sold in the country and the potential danger that this poses for Spanish users.

If the sustained use of cannabis can have very harmful effects on the health of regular users, this problem increases as the age of users decreases. Niesink et al. (2013) drew attention to the permanent psychological disorders that the recreational use of cannabis can cause in adolescents. In the case of Spain, as already described in the introduction, the prevalence of cannabis use among Spanish students aged 15 to 16 years is higher than the European average (EMCDDA, 2019). Indeed, the Spanish government's report (Delegación del Gobierno de España para el Plan Nacional sobre Drogas, 2019), based on the national system of drug addiction indicators (Sistema Estatal de Indicadores sobre Toxicomanías), found that among those aged under 18 years, cannabis was the drug responsible for almost all admissions to treatment for psychoactive substance use in outpatient centres, representing 96.6% of such admissions. Moreover, attenuated psychotic experiences have been analysed in adolescents in relation to cannabis use, with the finding that its consumption increased the risk of comorbid psychopathology (Fonseca-Pedrero, Lucas-Molina, Pérez-Albéniz, Inchausti & Ortuño-Sierra, 2020). Similar studies, also carried out in adolescents, have observed that symptoms such as depression or anxiety partially mediate between cannabis use and psychotic experiences (Bourque, Afzali, O'Leary-Barrett & Conrod, 2017; Reeves et al., 2014). However, attempts to establish an association between cannabis use and cognitive impairment in schizophrenia and first psychotic episodes have been hampered by the great disparity observed in the published results, thus preventing relevant conclusions from being reached in this regard (García Álvarez, Gomar, García-Portilla & Bobes, 2019). In short, the results of our study support the opinion of health officials that cannabis is not a harmless drug of abuse, but rather a highly dangerous substance with aggravating effects on certain groups.

CBN is a product of THC degradation. Therefore, its content in fresh cannabis is minimal. In fact, it is possible to estimate the freshness or "age" of cannabis products based on the relative concentration of CBN to THC (Ross et al., 1997). Thus, cannabis samples with a CBN/THC ratio below 0.013 would be less than six months old, and those with a ratio between 0.04 and 0.08 would be between one and two years old (Ross et al., 1997). By this criterion, only samples with very high potency could be considered fresh in our analysis, with an estimated age of between six months and one year. High potency samples would be between one and two years old and the rest of our samples (with medium, low or very low strength) would be at least two years old. Especially significant is the fact that the samples with high and medium potency, although they could not be considered fresh, presented THC values above 15%. These data corroborate the high potency of the cannabis samples that can be obtained in the Madrid region.

Certain organoleptic characteristics of cannabis such as its colour, elasticity and texture are commonly used by consumers to make a subjective estimate of the quality (strength) of the samples, with those samples with a sticky, elastic and dark-coloured texture considered to be of best quality. However, there have not been any previous studies analysing the possible relationship between the potency of the samples and their organoleptic characteristics objectively. Our results indicate that only in the case of very highly potent samples is there an association with a sticky texture and high elasticity. Interestingly, and contrary to the general belief among consumers (*Hachís: cómo reconocer su buena calidad* [Hashish: how to recognize good quality], 2017), it is the light brown samples and not the dark coloured samples that have the highest strength. In fact, the samples that simultaneously met the three characteristics (sticky texture, high elasticity and light brown colour) had significantly higher THC content than the other samples. However, it should be noted that there was a high percentages of samples with high potency which also presented dry texture, zero elasticity and dark brown colour. Therefore, with the exception of those samples with very high potency, we cannot conclude that texture, elasticity and colour are useful characteristics to use as criteria on which to base an estimate of the quality/strength of cannabis resin.

## Conclusions

The present study shows that most of the cannabis resin that can be bought in the Autonomous Community of Madrid contains over 15% THC. This finding, together with the low values of the CBD/THC ratio, shows that cannabis is not a harmless substance, and its use, both regular and occasional, represents a high health risk, especially in the case of Spanish adolescents, who use it at higher rates than in the rest of Europe. For this reason, the Spanish authorities should consider establishing systematic programs to monitor the strength of the cannabis made available to consumers and assess the negative consequences of high-potency cannabis use. Likewise, the sticky texture, high elasticity and light brown colour characteristics of the samples would only be useful for estimating the quality/strength of the cannabis resin when all three concur simultaneously.

## Contributions

Inmaculada Santos-Álvarez, Pilar Pérez-Lloret, Juncal González-Soriano and Manuel Pérez Moreno designed the study. Manuel Pérez Moreno acquired and analysed the samples. Inmaculada Santos-Álvarez, Manuel Pérez-Moreno and Pilar Pérez-Lloret wrote the first draft of the manuscript. Inmaculada Santos-Álvarez and Juncal González-Soriano analysed and interpreted the data and carried out the editing and critical review of the article. All authors approved the final document.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

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