CANNABIS OLIVE OIL: comparison among different preparation methods

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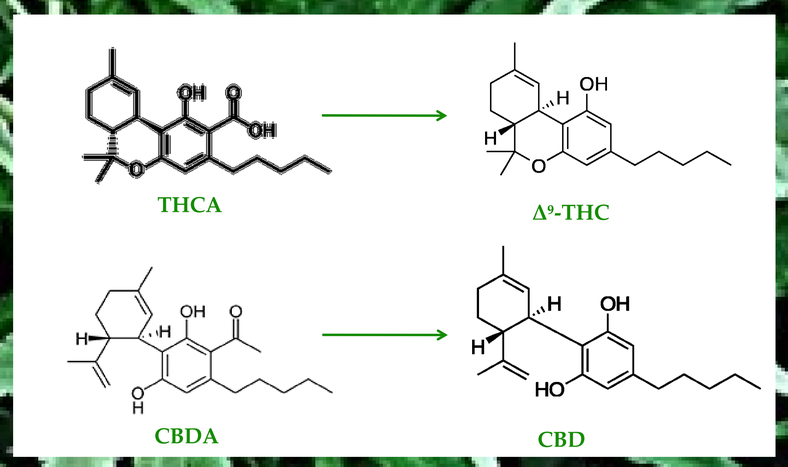
Introduction

The therapeutic properties of *Cannabis sativa* are widely recognized nowadays. Due to an increasing interest and the lack of authorized medicinal products, Italian pharmacists are involved in compounding Cannabis magistral preparations, mostly based on the olive oil extraction of cannabinoids from inflorescences. The main extracted components are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), and their corresponding acid, THCA and CBDA. A compendial procedure is not yet available, so methods proposed in scientific literature are followed to prepare Cannabis olive oils [1-4].

The methods most frequently used are four, based on maceration of vegetable materials in olive oil at high temperature. Two methods [1,2] don't involve a preliminary decarboxylation, step required to convert THCA and CBDA, molecules pharmacologically less active, into THC and CBD, the active neutral compounds. Decarboxylation can be obtained heating the plant materials at a temperature above 100°C before maceration in olive oil [3] or by sonication [4].

Aim of this work was to evaluate the Efficiency of Extraction (E.E.) of THC and CBD total contents in Cannabis olive oils compounded in the Italian pharmacies using four Cannabis varieties (Table 1) and following four different preparation conditions [1-4]. Over 3000 samples were analyzed in 2017 and 2018. The E.E. was calculated considering the standardized cannabinoid total content declared in the data sheet of each variety versus the same data obtained in analyzed samples. Cannabis olive oils are prepared with the recommended ratio cannabis/solvent 1g/10 mL. Due to the very low content (less than 0.2% w/w), CBD in Bedrocan and THC in Bedrolite was not considered.



Plant material

Bedrocan® is the brand name for the cultivar Cannabis sativa L. 'Afina'. It features 19-22% w/w THC with a CBD level below 1% (Bedrocan International).
Bediol® is the brand name for the cultivar Cannabis sativa L. 'Elida'. It has a balanced ratio of THC 6.3% and CBD 8%.
Bedrolite® is the brand name for the cultivar Cannabis sativa L. 'Talea'. It is a so-called CBD-only product, with less than 1% THC and 9% CBD.
FM2 is the brand name for Italian cultivation of Cannabis (Stabilimento Chimico Farmaceutico Militare, Florence). It has a balanced ratio of THC 5-8% and CBD 7.5-12%.

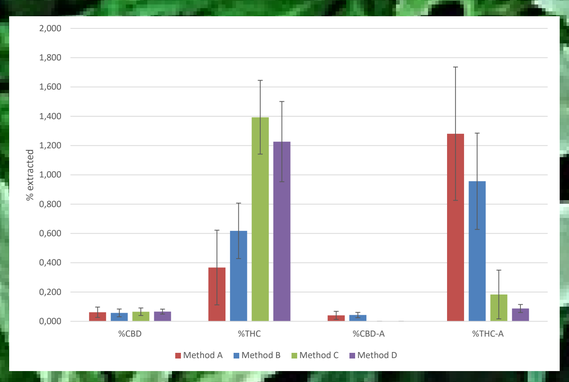
Extraction methods

- **Method A**: 5 g cannabis was put with 50 mL olive oil, then heated in water bath at about 98°C for 120 min. Before filtration, cooled down to room temperature. Filtrate by pressing [1].
- **Method B**: 5 g of cannabis inflorescence, fine powder, were placed in 50 mL of olive oil in a round bottom flask with a condenser and heated at 110 °C under magnetic stirring for 2 h. The mixture was gradually cooled down to room temperature over at least 2 h and then, it was paper filtered to obtain the final oil [2].
- **Method C**: cannabis flos was heated in a glass vial put in an oven at 115°C for 40 min. Then, 5 g of cannabis were finely grinded and added to 50 mL of olive oil. A mixer was used to further crumble the plant material. Then, the open beaker was put in a silicone-oil bath, pre-heated at fixed temperatures (160 °C). The mixture was stirred for 40 min and then immediately filtered to obtain the final oil [3].
- **Method D**: 1 g of superfine cannabis powder, prepared using mechanical grinding-activation in an energy intensive vibrational mill, was heated in a static oven at 145 °C for 30 min. For the extraction process, sonication (at 35 KHz for 30 min) was used. Total preparation time was 90 min [4].

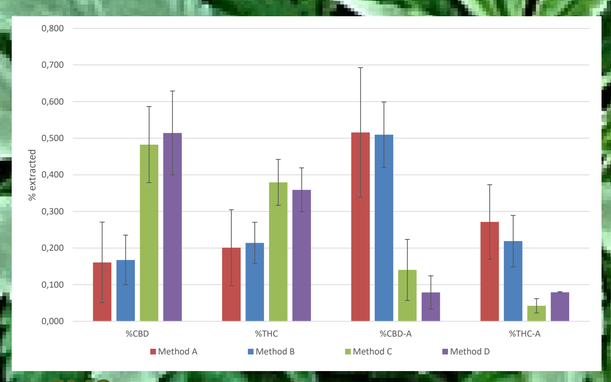
Results

Samples were prepared according the Methods A, B, C, D and results are reported in the hystograms.

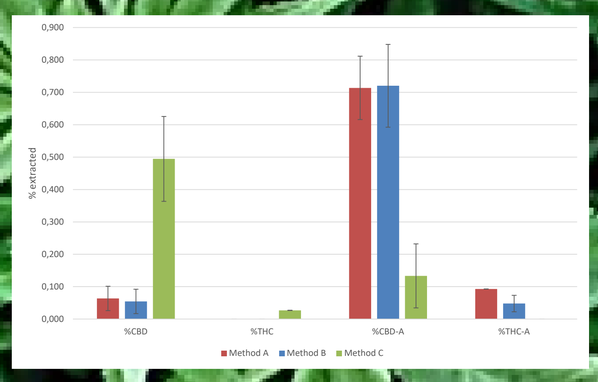
Bedrocan



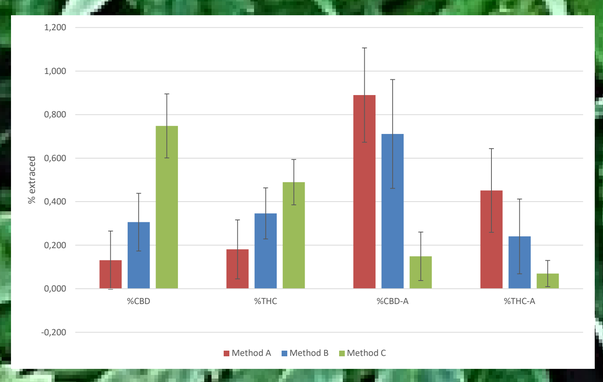
Bediol



Bedrolite



FM2



Samples obtained using Method A or B showed a high level of acidic forms, as expected. As the variability was quite high, details concerning the preparations were further asked to pharmacists and a certain number of variations to the original method were discovered. Therefore, some samples had to be discarded and the number of samples considered for the evaluation was reduced. After that, the variability of the cannabinoid content was consequently lower in many cases, as reported in the hystograms. This was more evident for Method A.
When decarboxylation came before extraction in oil (Method C and D), neutral forms were more abundant and both methods showed more reproducible values. Moreover, only in limited cases pharmacists introduced variations in the operative conditions. Despite inflorescence was subjected to heating at high temperature, the appearance of cannabinol (CBN), a degradation product, was limited and detected only in very few samples. When present, CBN was equal or below 0.04% w/w, therefore less than the admitted level of 0.1%.
The THC obtained values are significantly different among the methods, highlighting how the selected method is relevant for cannabinoids content. As expected, the THC content was the highest in the case of the Method C and D.
Considering results obtained for each method, values of total extraction and its efficiency are reported in Table 1.

Table 1 - Extraction efficiency (%) of CBD and THC measured in cannabis oil samples obtained using different cannabis varieties and preparation methods.

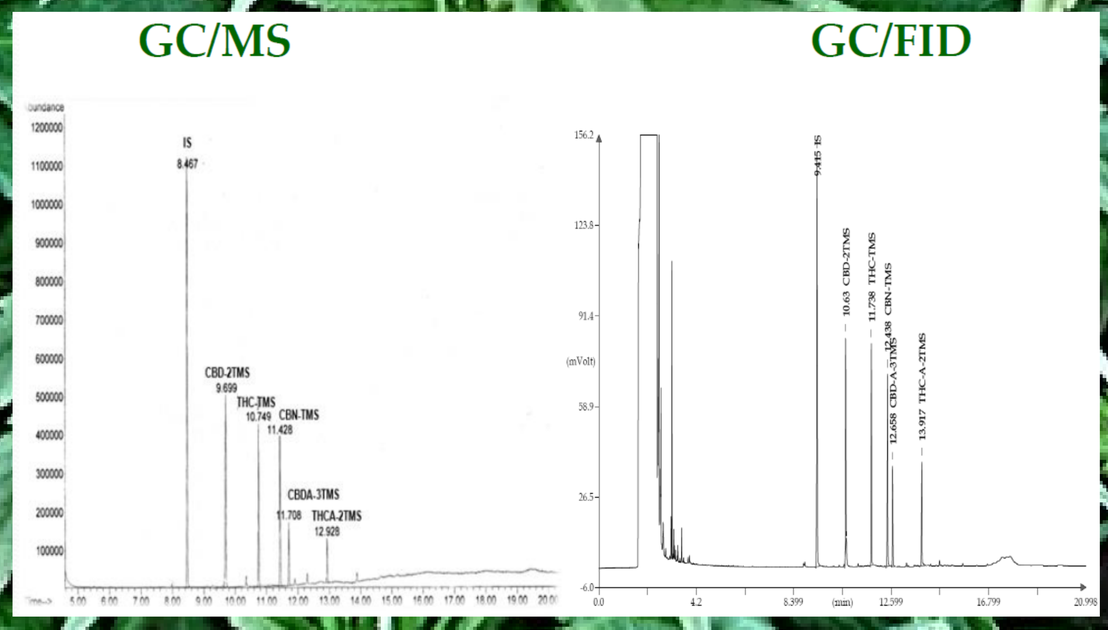
Preparation method	Cannabis sativa variety					
	Bedrocan (THC 2.2 w/w)	Bediol (CBD 0.8 w/w, THC 0.7 w/w)		Bedrolite (CBD 0.9 w/w)	FM2 (CBD 1.2 w/w, THC 0.8 w/w)	
	THC+THC-A	CBD+CBD-A	THC+THC-A	CBD+CBD-A	CBD+CBD-A	THC+THC-A
Method A (1)	71.5±23.5	83.9±53.1	72.8±34.9	82.7±60.1	84,1±60,0	85,0±55,1
Method B (2)	74.5±32.0	83.9±32.7	63.1±22.0	80.8±46.0	83,2±41,1	71,6±29,6
Method C (3)	71.6±19.0	79.0±27.9	62.8±16.4	73.2±32.8	74,8±21,5	69,9±20,5
Method D (4)	59.7±13.7	74.1±20.0	62.6±8.8	-	-	-

The E.E. of total THC and CBD in all cannabis varieties and for any preparation method resulted quite similar, slightly higher for CBD (almost always over 80%) than for THC (less than 75%). In case of varieties with similar CBD and THC content, Bediol and FM2, homogeneous E.E. values were observed. High variability was observed for the methods without decarboxylation (1,2).

Chromatographic conditions

Column	Agilent DB-5MSUI L: 30m ID: 0,25 mm Thickness: 0,25µm
Flow	1,1 ml / min
Inlet	280°C
Inlet mode	Split (30:1)
Oven	From 180°C to 300°C 10°C/min; 300 °C for 6,25 min
Solvent delay	4,5 min
Detector	300°C
Time of analysis	21 min

Column	Agilent DB-5MSUI L: 30m ID: 0,25 mm Thickness: 0,25µm
Flow	1,2 ml / min
Inlet	280°C
Inlet mode	Split
Oven	From 180°C to 300°C 10°C/min; 300 °C for 6,25 min
Detector	300°C
Time of analysis	21 min



Conclusions

The E.E. of total THC and CBD in all cannabis varieties and for any preparation method resulted quite similar. High variability was observed for the methods without decarboxylation (A,B). When decarboxylation came before extraction in oil neutral forms were more abundant and both methods showed more reproducible values. This leads to the conclusion that Methods C and D can be proposed as suitable methods for cannabinoid extraction.

References
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