

Original article

Cannabis Oil: chemical evaluation of an upcoming cannabis-based medicine

Luigi L Romano, Arno Hazekamp

Department of Pharmacy, University of Siena, Italy

Plant Metabolomics group, Institute of Biology, Leiden University, The Netherlands

Abstract

Concentrated cannabis extracts, also known as Cannabis oils because of their sticky and viscous appearance, are becoming increasingly popular among self-medicating patients as a claimed cure for cancer. In general, preparation methods for Cannabis oils are relatively simple and do not require particular instruments. The most well-known example of such a product is called ‘Simpson oil’. The purpose of the extraction, often followed by a solvent evaporation step, is to make cannabinoids and other beneficial components such as terpenes available in a highly concentrated form. Although various preparation methods have been recommended for Cannabis oils, so far no studies have reported on the chemical composition of such products.

Recognizing the need for more information on quality and safety issues regarding Cannabis oils, an analytical study was performed to compare several generally used preparation methods on the basis of content of cannabinoids, terpenes, and residual solvent components. Solvents used include ethanol, naphtha, petroleum ether, and olive oil. The obtained results are not intended to support or deny the therapeutic properties of these products, but may be useful for better understanding the experiences of self-medicating patients through chemical analysis of this popular medicine.

Keywords: cannabis oil, Rick Simpson oil, cancer, cannabinoids, terpenes

This article can be downloaded, printed and distributed freely for any non-commercial purposes, provided the original work is properly cited (see copyright info below). Available online at www.cannabis-med.org

Author's address: Arno Hazekamp, ahazekamp@rocketmail.com

Introduction

Cannabinoids exert palliative effects in cancer patients by reducing nausea, vomiting and pain, and by stimulating appetite [1]. In addition, preclinical evidence has shown cannabinoids to be capable, under some conditions, of inhibiting the development of cancer cells by various mechanisms of action, including apoptosis, inhibition of angiogenesis, and arresting the cell cycle [2,3]. As a result of such exciting findings, a growing number of videos and reports have appeared on the internet arguing that cannabis can cure cancer. But although research is on-going around the world, there is currently no solid clinical evidence to prove that cannabinoids - whether natural or synthetic - can effectively treat cancer in humans. It is therefore important

to be cautious when extrapolating preclinical results to patients.

Anecdotal reports on cannabis use have been historically helpful to provide hints on the biological processes controlled by the endocannabinoid system, and on the potential therapeutic benefits of cannabinoids. The antiemetic [4], appetite-enhancing [5], analgesic [6], and muscle-relaxant effects [7] and the therapeutic use of cannabinoids in Tourette’s syndrome [8] were all discovered or rediscovered in this manner. But although it is possible - and even desirable - that cannabis preparations exert an antineoplastic activity in, at least some, cancer patients, the current anecdotal evidence reported on this issue is still poor, and, unfortunately, remains far from supporting that cannabinoids are efficacious anticancer drugs for large patient popula-

tions [9]. It should be noted, however, that the potential effects of terpenes on cancer, either alone or in combination with cannabinoids, have not yet been addressed in laboratory studies. Indeed, the synergistic effect between cannabinoids and terpenes is often claimed to be the major difference between ‘holistic’ herbal preparations of cannabis, and products based on single cannabinoids [10]. Moreover, self-medicating patients often use extraction methods and/or administration forms that are quite different from conditions used in (pre)clinical studies, possibly resulting in different serum profiles of cannabinoids and their metabolites [11] and, consequently, in different therapeutic effects. Because of this gap between clinical research and real experiences, the curative potential of whole cannabis preparations for the treatment of different cancer types remains unclear.

In recent years an increasing number of patients have been using concentrated extracts of herbal cannabis, which, because of its sticky and viscous appearance, has become known as “Cannabis oil”. Among the self-medicating population, it is firmly believed that these products are capable of curing cancer, a claim that is backed up by numerous anecdotal patient stories. Cannabis oil is a concentrated extract obtained by solvent extraction of the buds or leaves of the cannabis plant. Various non-polar solvents have been recommended for this purpose, including petroleum ether, naphtha, alcohol and olive oil. The purpose of the extraction, often followed by a solvent evaporation step, is to make cannabinoids and other beneficial components such as terpenes available in a highly concentrated form. In general, preparation methods for Cannabis oil are relatively simple and do not require particular instruments. For this reason, people who have access to cannabis, either home grown or obtained from licensed pharmacies, dispensaries, coffee shops or the black market, may prepare it at home by themselves.

In particular, the captivating story of a former patient called Rick Simpson, a Canadian who claims to have cured his skin cancer through repeated topical application of Cannabis oil produced according to his own recipe, has received increasing attention. His detailed story is described on his website [12] and in a documentary film called “run from the cure” [13] where various cancer patients describe the therapeutic effects of ‘Simpson’ oil on their medical condition. In both the website and documentary, it is explained in detail how to prepare and administer the product. The method suggests the use of naphtha or petroleum ether as a solvent for the extraction, without specifying a particular quality or source. Both solvents are a mixture of petroleum hydrocarbons (PHCs), often available in a wide range of qualities. In general, petroleum ether and naphtha refer to very similar products, even though different names may be used around the world; e.g. in some countries naphtha is equivalent to diesel or kerosene fuel. As a result, extensive discussions on solvent choice can be found on web-forums. Following the success of Simpson oil, a number of related recipes

have sprung up, emphasizing small but significant changes to the original recipe. Examples include focusing on extraction with safer solvents such as ethanol [14], or preventing exposure to organic solvents altogether, by using olive oil [15].

Since cancer is a devastating disease that affects a large proportion of the world population, it causes some patients to seek alternative treatments outside the realm of modern medicine. With a growing interest in Cannabis oils for self-medication it is important not to overlook the importance of quality control and standardization. In this regard it should be noted that none of the production methods for Cannabis oil have been validated in published literature, and no reports have been made on the chemical composition of these products either. As a result, although many believe Cannabis oil may cure cancer, no one seems to know what is actually in it. Instead, the positive effects of Cannabis oil are based almost exclusively on case-reports by people who have used it. This paper evaluates the effects of preparation methods, and particularly the solvents used, on the final composition of the different Cannabis oils. The obtained results are not intended to support or deny the therapeutic properties of these products, but may be useful for better understanding the experiences of self-medicating patients through chemical analysis of this popular medicine.

Materials and Methods

Plant material

Cannabis plant material used in this study was of the variety ‘Bedrocan’ (19% THC w/w) and was obtained from Bedrocan BV (Veendam, The Netherlands) where it was cultivated under standardized conditions according to the requirements of Good Agricultural Practice (GAP). Only female flower tops were used (‘Cannabis Flos’). After harvest, the plant material was air-dried in the dark under constant temperature and humidity for 1 week. Dried flowers were manicured to remove leaves and stems, and finally cut in smaller pieces. The same cannabis material is officially dispensed through Dutch pharmacies under the medicinal cannabis program of the Netherlands, supervised by the Office of Medicinal Cannabis (OMC). The plant material was homogenized by grinding, and stored at -20°C until used.

Chemicals and solvents

Ethanol (HPLC grade), methanol (HPLC grade), acetic acid (analytical grade) and activated charcoal (analytical grade) were purchased from Sigma-Aldrich (Steinheim, Germany). Petroleum ether (boiling point 40-65°C; analytical grade) was purchased from Boom BV (Meppel, The Netherlands). Naphtha (light hydrotreated petroleum distillate; Coleman® fuel) was purchased from the Coleman Company (Wichita, USA). Olive oil (extra virgin quality) was purchased from a local grocery store. Deuterated chloroform (CDCl₃) was from Eurisotop (Gif-sur-Yvette, France). Pure ethanolic standards for delta-9-tetrahydrocannabinol (THC) and

delta-9-tetrahydrocannabinolic acid (THCA) were produced as previously described [16,17]. Cellulose

filter paper for filtration of extracts was from Whatman Ltd. (Maidstone, UK).

Table 1: Detailed description of the five different protocols used for preparation of Cannabis oils.

Preparation step	1) NAPHTHA	2) PETROLEUM ETHER	3) ETHANOL	4) OLIVE OIL I	5) OLIVE OIL II
CANNABIS (g)	5g	5g	5g	5g	10g
SOLVENT (mL)	Naphtha (200 mL)	Petroleum ether (200 mL)	Ethanol (200 mL)	Olive oil (20 mL) + water (70 mL)	Olive oil (100 mL)
EXTRACTION/ FILTRATION	Extraction #1: 5 g cannabis + 100 mL naphtha, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mL naphtha, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	Extraction #1: 5 g cannabis + 100 mL petr. ether, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mL petr. ether, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	Extraction #1: 5 g cannabis + 100 mL ethanol, agitate 20 min. (a) • Filtration with filter paper • Extraction #2: Same cannabis + 100 mL ethanol, agitate 20 min. (a) • Filtration with filter paper • Combine extracts	5g cannabis + 20 mL olive oil + 50 mL water. Heat in water bath ~98°C for 60 min. • Before filtration, let it stand to cool off. • Filtrate by pressing (b) • Rinse the plant material with 20 mL of hot water • Filtrate by pressing (b) • Combine extracts	10 g cannabis + 100 mL olive oil. Heat in water bath ~98°C for 120 min. • Before filtration, let it stand to cool off. • Filtrate by pressing (b)
EXTRACT CLEAN-UP	N/A	N/A	(optional): Filter extract over a column filled with activated charcoal	N/A	N/A
EVAPORATION/ SEPARATION	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Evaporate solvent in water bath ~98°C under stream of nitrogen gas	Let the solution stand to separate water and oil. Put it in the freezer (-20°C) overnight	N/A
RECONSTITUTION	Reconstitute residue with EtOH to 100 mL	Reconstitute residue with EtOH to 100 mL	Reconstitute residue with EtOH to 100 mL	Collect upper layer (oil) by pouring it off the frozen water layer	Collect the oil
EXTRACT CONCENTRATION (cannabis/solvent)	5 g/100 mL	5 g/100 mL	5 g/100 mL	5 g/20 mL	10 g/100 mL
DILUTION FACTOR FOR ANALYSIS	20x	20x	20x	100x	40x
FINAL CONCENTRATION (cannabis/solvent)	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL

a): agitate by using a shaking platform @ 120 rpm

b): separate oil from plant material by using a French coffee press

Effects of preheating

Preheating of cannabis samples has been recommended as a way to potentiate the final extract, i.e. to decarboxylate the acidic cannabinoids naturally present in cannabis plant material, such as THCA and CBDA, and turn them into their more potent counterparts such as THC and CBD [18,19]. Therefore, we tested two decarboxylation methods by heating cannabis plant material (1 g in an open glass vial) under two conditions: I) in a water bath at a low boil (temp. 98-100°C) for 5 min, and II) in an oven heated at 145°C for 30

min. Unheated samples were used as a control for these experiments. All experiments were done in duplicate. Subsequently, samples were extracted as previously described [20,21] and analyzed by HPLC and GC.

Preparation of concentrated extracts

Five different extraction protocols for the preparation of concentrates were assessed. Details are described in table 1. These included a naphtha (1) and a petroleum ether extraction (2) according to the procedure of Rick Simpson [12,13]; an ethanol extraction based on an

authoritative Dutch website on Cannabis oil [14]; and two olive oil extractions using different heating durations (4, 5) based on popular Youtube videos [15]. Chemically, naphtha and petroleum ether are very similar solvents, and sometimes hard to distinguish because of the many different qualities available. In the context of this study we selected an industrial quality naphtha that was sold as camping fuel (Coleman®) and contains added chemicals for improving stability, while the petroleum ether used was of laboratory quality, and represents a more pure and better characterized product. Both solvents may be purchased by inexperienced patients under the name naphtha or petroleum ether.

All preparation methods consisted of only a few simple steps, typically involving one or two extraction steps, separating plant material from solvent, and finally (in case of organic solvents) an evaporation step to produce a concentrate. For the ethanol extraction (3) we also tested the effect of filtration over activated charcoal, intended to remove chlorophyll which is strongly extracted by ethanol and may add an unpleasant 'green' flavour to the extract. Because the different extraction methods used different solvent-to-plant ratios, all extracts were finally diluted in ethanol to obtain a solvent-to-plant ratio of 2.5 mg/mL in order to allow direct chromatographic comparison of cannabinoid and terpene contents by high performance liquid chromatography (HPLC) and gas chromatography (GC).

GC/FID analysis

Because of the heat applied during injection and separation, GC is not able to show the presence of acidic cannabinoids without sample derivatization. As a result, GC reveals the total cannabinoid content (acidic + neutral cannabinoids) after decarboxylation, only. However, terpenes can be efficiently analyzed by GC. Therefore, an Agilent GC 6890 series (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a 7683 autosampler and flame ionization detector (FID) was used for the analysis of cannabis terpenes as previously described [20,21]. The instrument was equipped with a DB5 capillary column (30 m length, 0.25 mm internal diameter, film thickness 0.25 μm ; J&W Scientific Inc., Folsom, CA, USA). The injector temperature was 230°C, with an injection volume of 4 μL , a split ratio of 1:120 and a carrier gas (N₂) flow rate of 1.2 mL/min. The temperature gradient started at 60°C and increased at a rate of 3°C/min until 240°C which was held for 5 min resulting in a total run time of 65 min. The FID temperature was set to 250°C. The GC was controlled by Agilent GC Chemstation software version B.04.01

HPLC analysis

Cannabinoid profiles were studied in more detail by HPLC, which enables the differentiation of acidic cannabinoids (THCA, CBDA etc.) and their neutral analogues (THC, CBD etc.). Analyses were carried out using an Agilent (Agilent Technologies Inc., Santa

Clara, CA, USA) 1200 series HPLC system, consisting of a G1310A pump, an G1322A solvent degasser, and a G1329A autosampler. Full spectra were recorded in the range of 200-400 nm using a G1315D photodiode-array (PDA) detector. Chromatographic separation was achieved using a Phenomenex C18 column (type Kinetex, 2.6 μm , 3 x 100 mm). Equipment control, data acquisition and integration were performed with Agilent Chemstation software. The mobile phase consisted of methanol and water, acidified with 25 mM formic acid. Initial setting was 75% methanol (v/v), which was linearly increased to 100% methanol over 10 min. After maintaining this condition for 1 min, the column was re-equilibrated under initial conditions for 4 min, resulting in a total runtime of 15 min. The flow-rate was set to 0.5 mL/min, the injection volume was 2 μL , and the detection wavelength was 228 nm. All experiments were carried out at a column temperature of 40 °C.

NMR analysis

Proton Nuclear Magnetic Resonance (¹H-NMR) analysis for detection of solvent residues was performed by dissolving sample aliquots in deuterated chloroform. Spectra were recorded on a Bruker DPX 300MHz spectrometer, as previously described [17].

Results and Discussion

Effects of preheating

In the cannabis plant, cannabinoids are biosynthesized as their acidic forms, characterized by the presence of a carboxyl group attached to the phenolic ring. Acidic cannabinoids can be rapidly converted into their 'neutral' analogues under the influence of heat or extended storage [18], which causes loss of the relatively unstable carboxyl group in the form of carbon dioxide (decarboxylation). Preparation of cannabis oil, mainly intended for oral use, usually involves temperatures that are relatively low compared to other forms of administration where heating of the material is typically performed at much higher temperatures (e.g. smoking, vaporizing or baking). For a more thorough decarboxylation, preheating of herbal cannabis before preparation of cannabis oil has been suggested, for example by placing the cannabis in an oven.

Besides cannabinoids, the cannabis plant contains a range of terpenes, which are the volatile compounds that give cannabis its distinct smell and may act synergistically with cannabinoids [10]. Although preheating the plant material may release more of the known active (neutral) cannabinoids, it may simultaneously also cause loss by degradation or evaporation of components such as terpenes. Our tests were intended to better clarify the balance between desired decarboxylation and unwanted degradation. Unheated cannabis material was analyzed as a control.

Figure 1A shows the cannabinoid profile of the decarboxylated samples, obtained by HPLC analysis. The

mild water bath treatment did not lead to significant changes in the acidic-to-neutral cannabinoid ratio. In contrast, the oven treatment resulted in a complete decarboxylation of the major cannabinoids detected. THCA, cannabigerolic acid (CBGA) and cannabichromenic acid (CBCA) had all fully converted into THC, cannabigerol (CBG) and cannabichromene (CBC), respectively. Further conversion of THC into its' main degradation product cannabinol (CBN) only took place to a small degree during the oven treatment. Figures 1B and 1C show the terpene profile acquired in our decarboxylated samples using GC. Compared to the untreated control, monoterpenes (the most volatile class of terpenes) were reduced to about half of their original levels even after exposing the plant material to boiling water for just 5 min. After the more intense oven treatment, only small traces of the monoterpenes terpineol, myrcene and terpinolene could still be detected. As may be expected, the less volatile sesquiterpenes were more resistant to the mild treatment with the water bath. However, most of them were lost in the oven treatment, and only traces of gamma-cadinene and eudesma-3,7(11)-diene remained.

These data indicate that significant decarboxylation of the major cannabinoid acids occurs only by exposure to higher temperatures for extended time (oven at 145°C for 30 min), which is in agreement with previous studies [18,22]. However, under these conditions all major terpenes present were affected by significant evaporation. Although milder decarboxylation using a boiling water bath may be efficient when applied for longer time [22], the terpene profile already changes significantly after only 5 min of treatment. For this reason, all further experiments were carried out without application of a preheating step.

Analysis of the extracts: cannabinoid and terpene content

Analysis by HPLC to reveal the ratio between acidic and neutral cannabinoids in the different extracts was limited to the main cannabinoids THCA and THC. Results are shown in Figure 2A. Most extracts contained only a small proportion of THC (5-10% of total THCA + THC content), as a result of the relatively low heat of max. 100°C applied during the evaporation (protocol 1-3) or extraction (protocol 4-5) step. A notable exception was the naphtha extract, which was found to contain 33% of total THCA + THC content present in the form of THC. This is remarkable because the extract prepared with petroleum ether did not show the same composition, even though both solvents are chemically quite similar. Perhaps added chemicals (e.g. for stability) in the naphtha used in this study may be responsible for the observed difference.

Analysis of the extracts by GC indicated that the major components present in the cannabis material used were the monoterpenes beta-pinene, myrcene, beta-phellandrene, cis-ocimene, terpinolene and terpineol, and the sesquiterpenes beta-caryophyllene, humulene, delta-guaiene, gamma-cadinene, eudesma-3,7(11)-

diene and elemene. This is in agreement with previous reports on cannabis variety 'Bedrocan' [20,21].

The extraction solvents showed comparable efficiency for extracting terpenes, with the notable exception of naphtha (Figure 2B and 2C). While this solvent generally extracted terpenes less efficiently than the other solvents, several terpenes could not be detected at all in the naphtha extract. It is not known whether (i) these components were not extracted from the plant material, (ii) were degraded or evaporated during the extraction protocol, or (iii) GC retention times for these components were changed as a result of interaction with solvent components. Interestingly, the use of petroleum ether (chemically very similar to naphtha) did not show the same absence of components.

The use of olive oil as extraction solvent was found to be most beneficial based on the fact that it extracted higher amounts of terpenes than the other solvents/methods, especially when using an extended heating time (120 min; protocol 5). This may be explained by the highly non-polar but also non-volatile character of olive oil, resulting in a good solubilization of terpenes while limiting their loss by evaporation.

Treatment of the ethanolic extract with activated charcoal, intended to remove chlorophyll, resulted in a considerable reduction of cannabinoid content (~50%) as well as all other sample components (data not shown). For this reason, the use of charcoal should not be recommended and was not further evaluated in our study.

Residual solvent testing

Naphtha and petroleum ether are mixtures of various hydrocarbon solvents with a range of boiling points, typically between 30 - 200°C. All the solvent components should be considered harmful and flammable, and some of them, such as hexane and benzene, may be neurotoxic. Both naphtha and petroleum ether are considered potential cancer hazards according to their respective Material Safety Data Sheets (MSDS) provided by manufacturers. Moreover, products sold as naphtha may contain added impurities (e.g. to increase stability) which may have harmful properties of their own [23]. For these reasons, the naphtha and petroleum ether extracts were analyzed for residual solvent content.

Analysis by GC as well as NMR revealed significant residues of petroleum hydrocarbons (PHCs) in the naphtha and petroleum ether extracts. As may be expected, mainly PHCs with a higher boiling point (as indicated by longer GC retention times) were detected, as they are more resistant to the evaporation procedure used (Figure 3A). In the naphtha extract, based on GC peak areas, the content of naphtha residue was roughly similar to the total content of terpenes remaining in the extract (Figure 3B).

Reconfirmation using an actual patient sample

In order to confirm our experimental results, we also analyzed a sample provided by a patient in the Netherlands who produced his own cannabis oil using

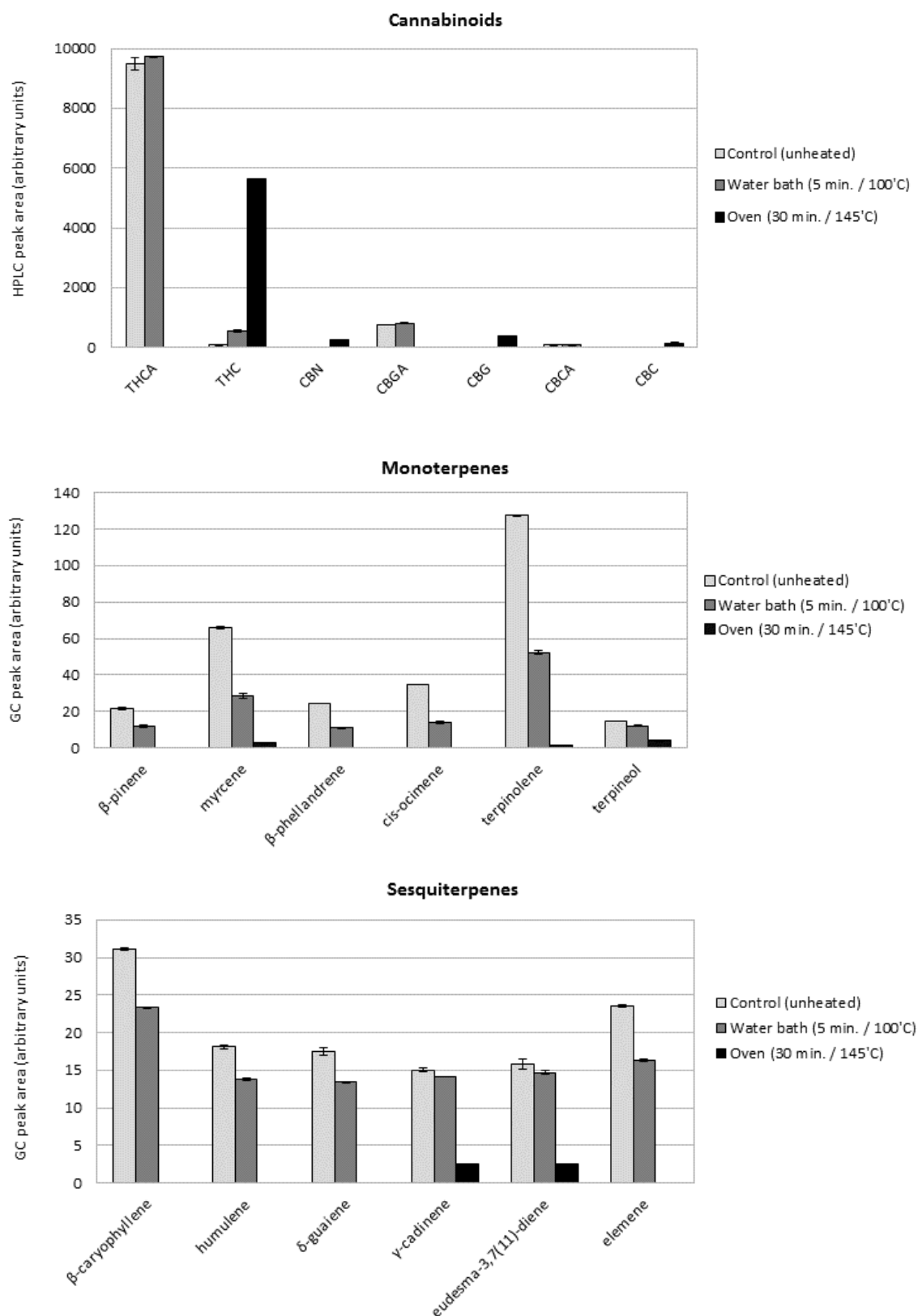


Figure 1: (A) Effect of (pre-)heating on the cannabinoid (HPLC analysis), (B) monoterpene and (C) sesquiterpene composition (GC analysis) of herbal cannabis material. (THCA: tetrahydrocannabinolic acid; THC: tetrahydrocannabinol; CBN: cannabinoil; CBGA: cannabigerolic acid; CBG: cannabigerol; CBCA: cannabichromenic acid; CBC: cannabichromene)

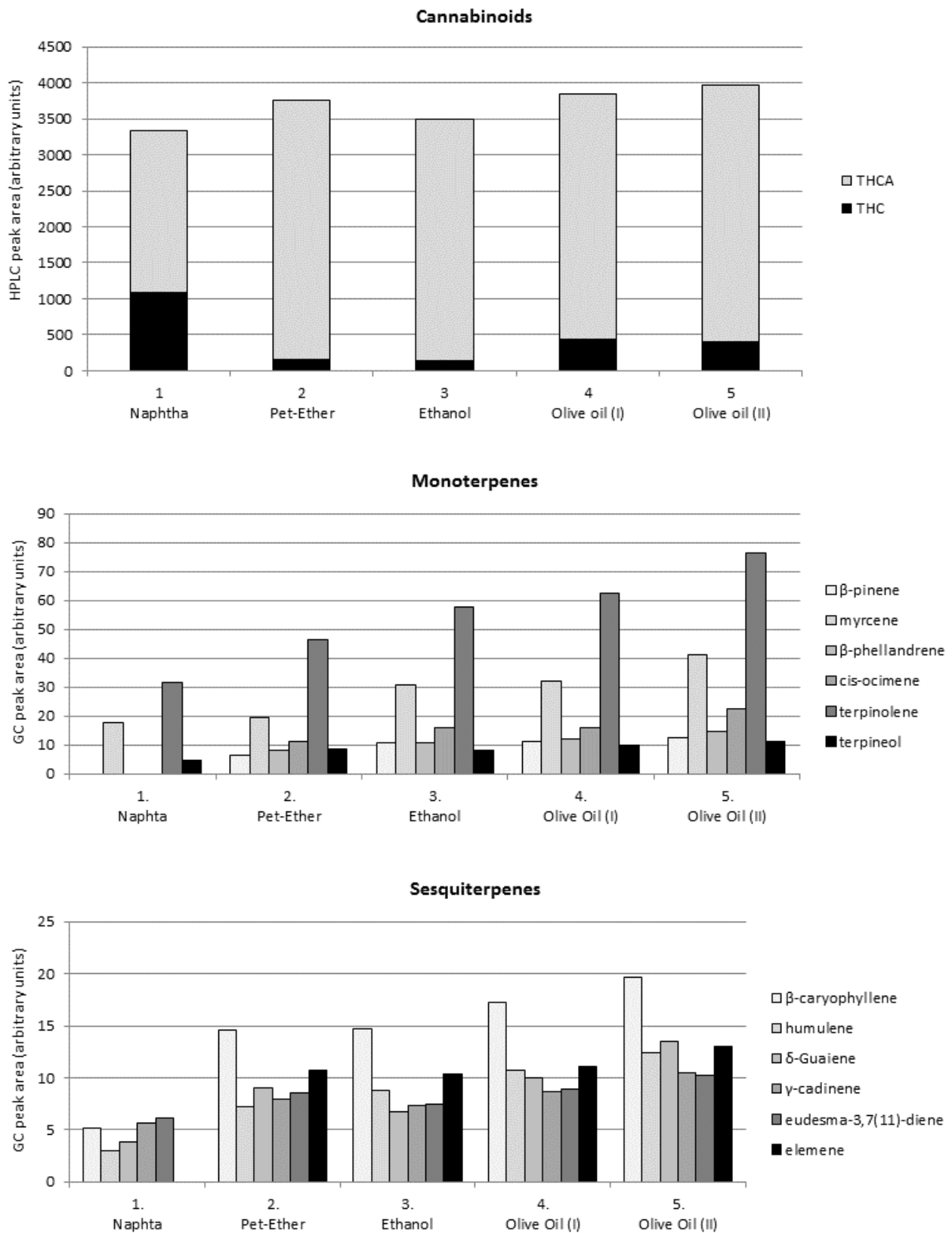


Figure 2: (A) Effect of five different preparation methods on the cannabinoid (HPLC analysis), monoterpene and sesquiterpene composition (GC analysis) of concentrated cannabis extracts.

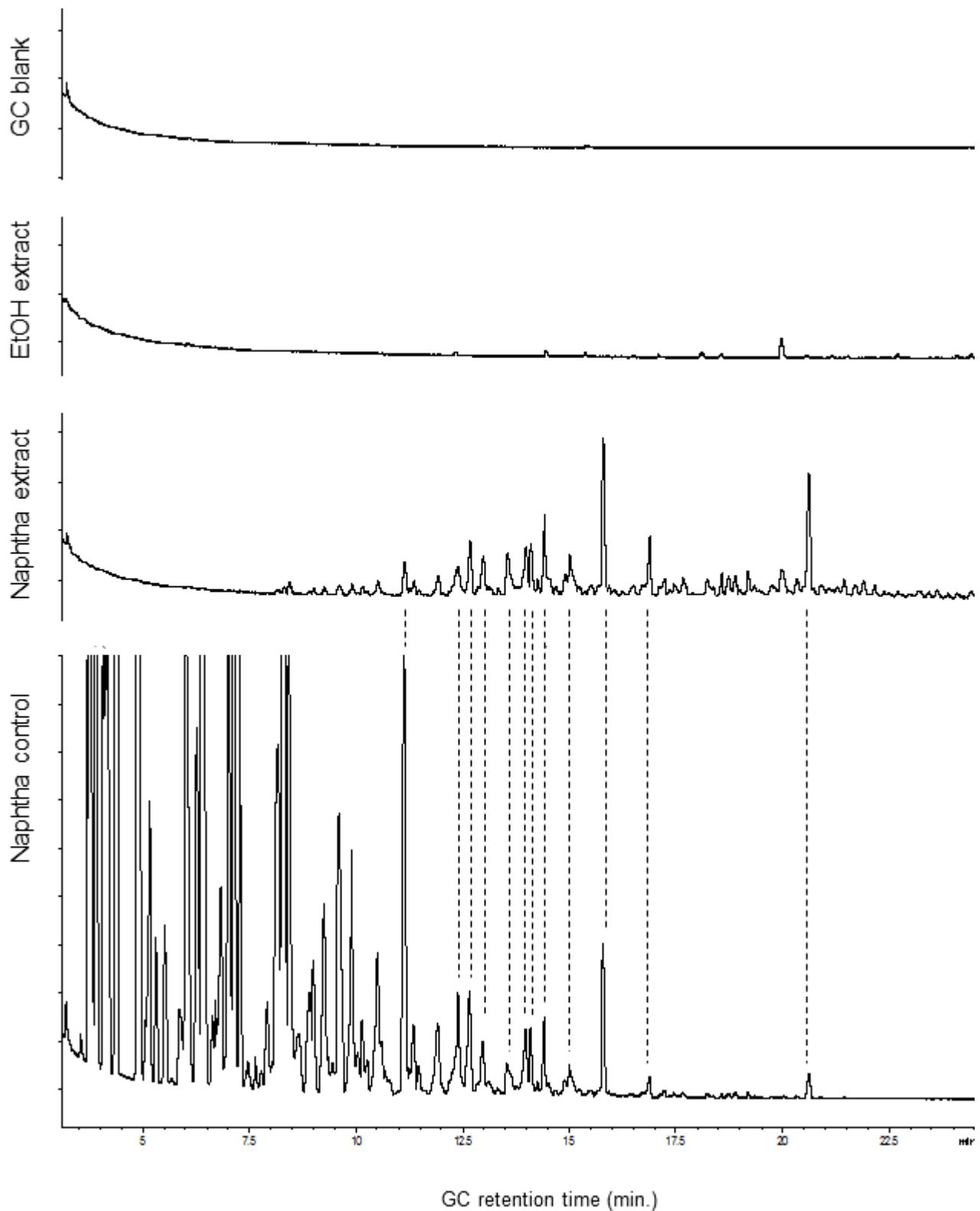


Figure 3a: Residual naphtha solvent components present in the naphtha extract as indicated by GC analysis. Dotted lines are added for easier comparison. All chromatograms are shown at the same vertical scaling.

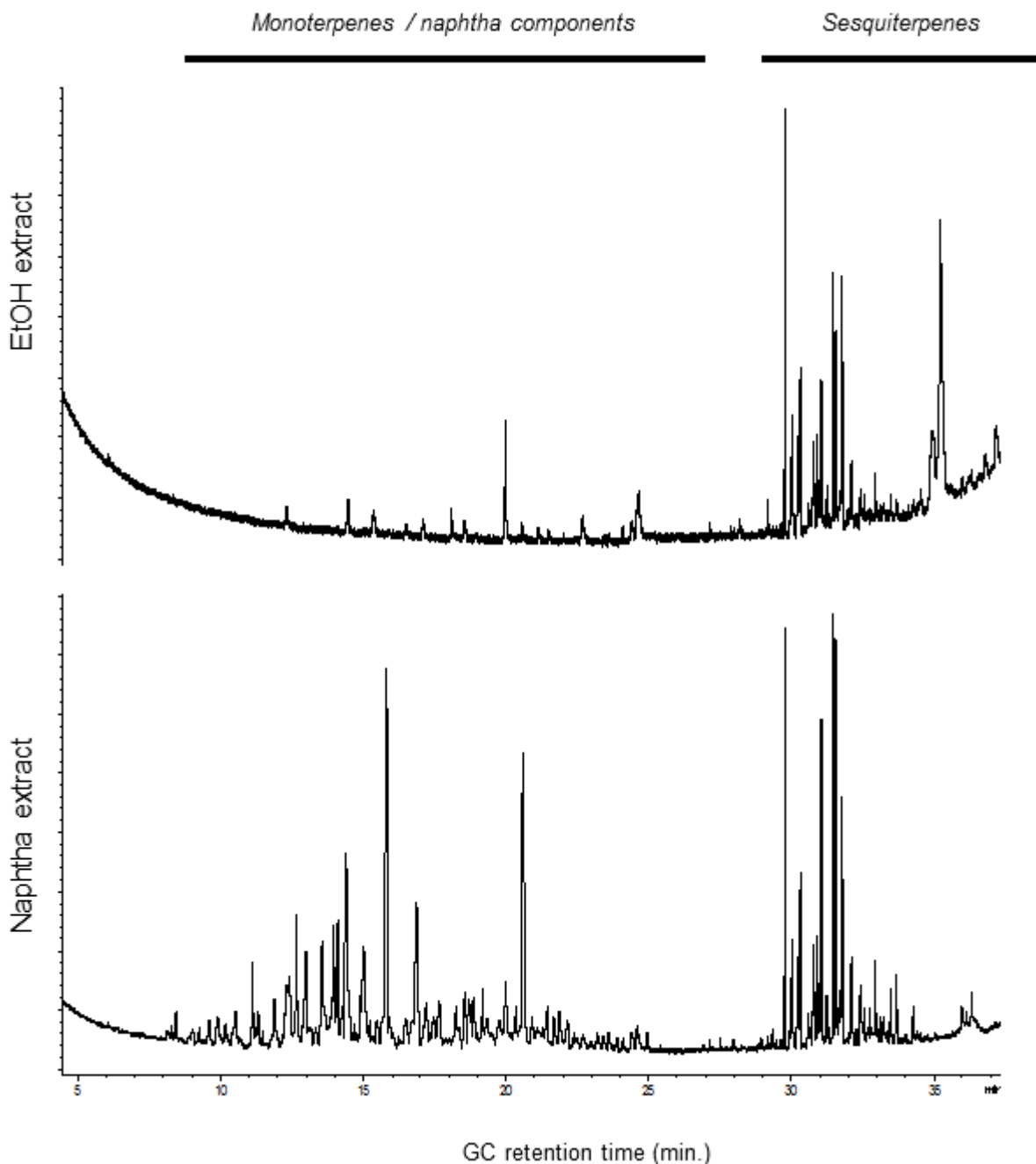


Figure 3b: GC analysis showing the same ethanol and naphtha extracts as above (Fig. 3a), but now using a larger time scale to compare total peak area of naphtha components to the sesquiterpenes present in these samples.

Bedrocan® cannabis and following the Simpson method as described in the internet. The patient was a 50 year old male suffering from cancer of the (left) tonsil and the tongue. The analytical results (data not shown) were equivalent to our lab experiments described above, confirming the residual presence of PHCs at significant concentrations in a product that is intended for self-medication of cancer.

Conclusions

Concentrated cannabis extracts, also known as Cannabis oils, are increasingly mentioned by self-medicating patients as a cure for cancer. Despite this growing popularity, so far no studies have been reported on the chemical composition or on the different preparation methods of such products. Recognizing the need for more information on quality and safety issues regard-

ing Cannabis oils, the small study presented here compared on the basis of cannabinoid, terpene, and residual solvent content a few generally used recipes for preparation of Cannabis oils.

Based on the results of our preheating experiments, comparing a mild water bath treatment to more intense heating in an oven, it can be concluded that it is not feasible to perform decarboxylation of cannabinoids, without significant loss of terpene components. This is particularly important because of the fact that users of Cannabis oils often claim the holistic nature of cannabis components to be responsible for its therapeutic effects. Retaining the full spectrum of terpenes present in fresh cannabis material should therefore be a major focus during optimal Cannabis oil production.

When comparing five methods of Cannabis oil preparation, some interesting differences were observed between the resulting extracts. Specifically the preparation method described by Rick Simpson has attracted quite a following of self-medicating patients. This method favours the use of naphtha as solvent for cannabinoid extraction, without specifying issues regarding quality or safety. According to the Simpson website: "All these solvents [...] are poisonous in nature, but if you follow these instructions solvent residue in the finished oil is not a concern. [...] Even if there was a trace amount of solvent residue remaining, the oil itself would act upon it to neutralize any harmful poisonous effect." [13]. In other words, the curative properties are considered to be strong enough to counteract any and all potential negative effects caused by residual solvents. Chemical analysis of our laboratory samples, as well as a sample obtained from a patient, showed that the heavy fraction (components with high boiling point) of naphtha indeed remains in the extract despite the recommended evaporation step. Based on GC-FID peak areas, the total content of PCHs roughly equalled the total content of terpenes present in the extract. The potential harmful effects of these solvent residues have been discussed above.

It should be noted that as a result of sample viscosity, the more concentrated an extract becomes, the more difficult it will be to remove the residual solvent from it. In such a case, applying more heat will increase evaporation, but simultaneously more terpene components will be lost as well. Especially under conditions where Cannabis oil is prepared by simple household methods, there will always be a trade-off between residual solvents and terpene content. For this reason, the use of non-toxic solvents should always be advised, so that potential residues are not harmful to health.

As extraction solvents for the production of Cannabis oils, ethanol and olive oil were shown to perform much better, extracting all terpenes and cannabinoids tested very efficiently. Additionally, these solvents are not harmful. Unfortunately, pure ethanol efficiently extracts chlorophyll from cannabis, which will give the final extract a distinct green colour, and often unpleasant taste. Removing chlorophyll by filtering the ethanol extract over activated charcoal was found to be very

effective, but it also removed a large proportion of cannabinoids and terpenes, and is therefore not advised. Additionally, in most countries consumption-grade ethanol is an expensive solvent, as a result of added tax on alcohol products.

Of the solvents tested, this leaves olive oil as the most optimal choice for preparation of Cannabis oils for self-medication. Olive oil is cheap, not flammable or toxic, and the oil needs to be heated up only to the boiling point of water (by placing a glass container with the product in a pan of boiling water) so no overheating of the oil may occur. After cooling down and filtering the oil, e.g. by using a French coffee press, the product is immediately ready for consumption. As a trade-off, however, olive oil extract cannot be concentrated by evaporation, which means patients will need to consume a larger volume of it in order to get the same therapeutic effects. In a follow-up study on the use of Cannabis oils, there should be more focus on the characteristics and motivations of those who use it for self-medication.

Acknowledgements

LR was funded for this research by the Puglia (Italy) regional government with a "Ritorno al futuro" grant. AH is the head of Research and Development at Bedrocan BV, the Netherlands.

References

1. Guzmán M. Cannabinoids: potential anticancer agents. *Nat. Rev. Cancer*. 2003; 3(10): 745-755.
2. Calvaruso G, Pellerito O, Notaro A, Giuliano M. Cannabinoid-associated cell death mechanisms in tumor models (review). *Int. J. Oncol*. 2012; 41(2): 407-413.
3. Velasco G, Sánchez C, Guzmán M. Towards the use of cannabinoids as antitumour agents. *Nat. Rev. Cancer* 2012; 12(6): 436-444.
4. Dansak DA. Cannabis as an antiemetic and appetite stimulant in cancer patients. *McFarland & Co.: Jefferson, NC*, 1997; pp 69-83.
5. Plasse TF, Gorter RW, Krasnow SH, Lane M, Shepard KV, Wadleigh RG. Recent clinical experience with dronabinol. *Pharmacol. Biochem. Behav*. 1991; 40(3): 695-700.
6. Noyes R, Baram DA. Cannabis analgesia. *Comprehensive Psychiatry* 1974; 15(6): 531-535.
7. Clifford DB. Tetrahydrocannabinol for tremor in multiple sclerosis. *Ann. Neurol* 1983; 13(6): 669-671.
8. Müller-Vahl KR, Kolbe H, Schneider U, Emrich HM. Cannabis in movement disorders. *Forsch. Komplementärmed*. 1999; 6 (Suppl 3): 23-27.
9. Guzman M 2012. Do cannabinoids cure cancer? IACM website: <http://www.cannabis-med.org/index.php?tpl=faq&red=faqlist&id=274&lng=en>
10. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br. J. Pharmacol*. 2011; 163: 1344-1364.

11. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharmacokin.* 2003; 42(4): 327-360.
12. Simpson R. 2008. Documentary film: Run from the cure. Available on youtube. http://www.youtube.com/watch?v=0psJhQHK_GI
13. Simpson R. 2013: Rick Simpson official website: <http://phoenixtears.ca/>
14. Bruining W. 2013. Wernard Bruining. Mediwiet [Medi-weed] official website: <http://www.mediwiet.nl>
15. Dr. Diane. 2013. Youtube instruction video: <http://www.youtube.com/watch?v=vs66uyiH968>
16. Hazekamp A, Simons R, Peltenburg-Looman A, Sengers M, Van Zweden R, Verpoorte R. Preparative isolation of cannabinoids from *Cannabis sativa* by centrifugal partition chromatography. *J. Liq. Chrom. Rel. Technol.* 2004; 27: 2421-2439.
17. Hazekamp A, Choi YH, Verpoorte R. Quantitative analysis of cannabinoids from *Cannabis sativa* using ¹H-NMR. *Chem. Pharm. Bull.* 2004; 52(6): 718-721.
18. Veress T, Szanto JI, Leisztner L. Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation in an open reactor. *J. Chromatogr.* 1990; 520: 339-347.
19. Hazekamp A, Bastola, K Rashidi H, Bender J, Verpoorte R. Cannabis tea revisited: A systematic evaluation of the cannabinoid composition of cannabis tea. *J. Ethnopharm.* 2007; 113: 85-90.
20. Fishedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R. Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochem.* 2010; 71(17-18): 2058-2073.
21. Hazekamp A, Fishedick JT. Cannabis - from cultivar to chemovar. Towards a better definition of cannabis potency. *Drug Testing and Analysis* 2012; 4: 660-667.
22. Fishedick 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: 421-426.
23. IARC 1989: WHO International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 47. <http://monographs.iarc.fr/ENG/Monographs/vol47/mono47.pdf>