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Variation in the compositions of cannabinoid and terpenoids in *Cannabis sativa* derived from inflorescence position along the stem and extraction methods

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ABSTRACT

In the last decade, recognition of the therapeutic abilities of *Cannabis sativa* has risen, along with the need to standardize its products. Standardization requires grading the methods for growing the plant and extracting the active compounds accumulated in its inflorescence. We explored the results of different methods used today and their effect on the levels of compounds extracted from inflorescences positioned along the *C. sativa* flowering stem. The polarity of the solvent used for the extraction, drying processes and separation methods influenced the chemical composition of the extract. However, regardless of extraction and analytical methods applied, the amounts of cannabinoids and terpenoids in the inflorescences decreased with the position of the sampled inflorescence from top to bottom of the flowering stem. These results have significant implications for the development of growth protocols for *C. sativa* cultivation and flower extraction methods to standardize cannabis-based products.

1. Introduction

Cannabis sativa is known for its mind-altering properties as well as for its beneficial effects; it is routinely used to treat patients with various medical indications. Marijuana has been used for its stimulating (or relaxing) properties since the dawn of man (Vincent et al., 1983; Zias et al., 1993; Merrillees, 1962). Besides being an intoxicating substance, the active compounds of *cannabis* are also indicated for their therapeutic effects in different diseases and disorders. Among the physical difficulties that *cannabis* has been reported to ease are eating disorders such as obesity, anorexia and emesis (Gelfand and Cannon, 2006; Patel and Pathak, 2007), diabetes (Penner et al., 2013; Weiss et al., 2006), pain modulation (Liang et al., 2004; Holdcroft et al., 1997) and multiple sclerosis-related pain (Iskedjian et al., 2007), inflammation (Croci and Zarini, 2007), neurodegenerative disorders such as Parkinson's disease (Alsausa del Valle, 2006; Lastres-Becker and Fernandez-Ruiz, 2003), Alzheimer's disease (Bachurin, 2003; Eubanks et al., 2006; Campbell and Gowran, 2007), Huntington's disease (Luvone et al., 2009; Sagredo et al., 2012), epilepsy (Porter and Jacobson, 2013; Devinsky et al., 2017), and pain in cancer patients (Hall et al., 2005; Herman et al., 1979; Hutcheon et al., 1983; Ungerleider et al., 1982).

More than 500 phytochemicals have been detected in *cannabis* strains to date (Aizpurua-Olaizola et al., 2016). Due to the large number of compounds and the need for standardized treatment of patients with respect to both composition and dosage, the use of *cannabis* should be standardized. Hence, both qualitative and quantitative knowledge of its phytochemical composition and production in the plant, and optimal methods of extraction are needed. Among *cannabis* compounds, phytocannabinoids have been most studied for their suggested therapeutic activity. About 113 different cannabinoids have been reported (Aizpurua-Olaizola et al., 2016; ElSohly and Gul, 2014; Ahmed et al., 2015). Of these, dronabinol (Δ^9-tetrahydrocannabinol, THC) and cannabidiol (CBD) are the most well-known, and have been defined as the most active phytocannabinoids (Mechoulam et al., 1970; Mechoulam and Gaoni, 1965; Mechoulam et al., 2002).

Another group of compounds in *C. sativa* is the terpenes (ElSohly and Slade, 2005). These are the main contributors to the plant's unique aroma. Terpenes have also been suggested to have a complementary effect to that of the phytocannabinoids. The function of terpenes in the different therapeutic processes is not yet understood, but their significant role is starting to be appreciated (Russo, 2011; Izzo et al., 2012). Although their total amount recovered from *C. sativa* is about tenth of that of cannabinoids extracted from the same plant, it is suggested that even a small amount of terpenes significantly affects the activity of cannabinoids (Russo, 2011). In general, three main terpene

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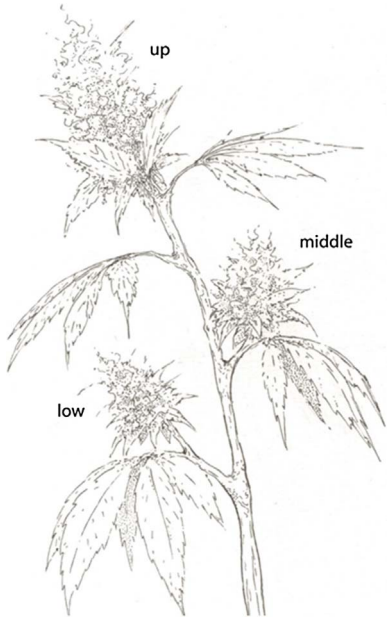

Fig. 1. Graphic illustration of the *Cannabis sativa* flowering stem and positions of the inflorescences. Sampling locations are marked up, middle and low in accordance to their position along the *cannabis* flowering stem (illustrated by Omer Koltai).

groups have been identified in *C. sativa* oil—monoterpenes, sesquiterpenes and terpene alcohols (Russo, 2011). Monoterpenes and terpene alcohols are highly volatile (El-Zaeddi et al., 2016). Sesquiterpenes are susceptible to degradation and show instability when exposed to the degradative action of air/oxygen and high temperature (Nigam and Levi, 1962; Turek and Stintzing, 2013; Hădărugă et al., 2014).

The high complexity of compounds accumulated in the inflorescence poses a significant challenge for standardization of *cannabis*-based products. One source of variation derives from the fact that different active compounds produced by the plant have different chemo-affinities; the various extraction methods therefore tend to produce different compositions with varied amounts of cannabinoids and terpenoids in the concentrate. Another source of variation in chemical composition that has been somewhat neglected is the effect of the position of the inflorescence along the flowering stem. Notably, during plant growth, artificial light is projected from above; thus, different inflorescences along the flowering stem are exposed to different amounts of light.

With the aim of specifying the different parameters that must be controlled for the production of standardized *cannabis* extract, we explored the implications of decisions taken during the extraction and analytical separation processes. We report the effects of several parameters on the quality and quantity of the different extracted and detected phytochemicals. These parameters were: (a) the position along the flowering stem of the sampled inflorescence, (b) the solvent chosen for extraction, from polar to non-polar, (c) the extract-drying processes, and (d) the extract-separation method—gas chromatography or high-pressure liquid chromatography.

2. Materials and methods

2.1. Materials

Cannabis plants (line CS12) were grown in 5-L pots. The planting bed was composed of 80% coconut slivers and 20% sifted tuff (0.8 cm grain size). Natural compost nutrient was added to 10% of the total bed volume. In the first 6 weeks, the plants were grown under an indoor vegetative light cycle of 20 h of light from a T5-36 W (fluorescent tube).

Table 1
List of all compounds detected in the *C. sativa* inflorescences using different extraction methods and analyzed by GC–MS. EtOH – polar extraction using ethanol as the solvent; Hex:EtOH – mixed polarity extraction, using *n*-hexane and ethanol (7:3, v/v); Hex – non-polar extraction with *n*-hexane. RT – retention time, in minutes. Calculated amounts are reported in mg mL^{-1}.

Compound name	EtOH	Hex:EtOH	Hex
α-Pinene	0.13	0.15	0.23
β-Pinene	0.47	0.4	0.76
β-Phellandrene	0	0.18	0.21
β-Pinene	0.78	0.64	1.21
β-Myrcene	2.32	1.82	3.2
alkane	0	0.12	0.14
α-Phellandrene	0.22	0.08	0.73
3-Carene	0.34	0.32	0.54
γ-Terpinene	1.6	1.43	2.39
β-Ocimene	2.69	2.23	3.42
alkane	0	0.42	0.4
Terpinene	0.2	0.1	0.35
alkane	0	0.14	0.15
ketone	0	0.05	0.05
2-Carene	4	2.65	1.7
Fenchol	0.1	0.1	0.16
ketone	0.27	0.23	0.09
ketone	0.48	0.13	0.06
benzenemethanol	0.32	0.25	0
α-Terpineol	0.21	0.26	0.38
alkane	0	0.3	0.41
alcohol	0.33	0.11	0
ascaridol	0.18	0.28	0.06
benzenediethylmethyl	0.09	0.6	0.86
alkane	0	0.33	0.35
ketone	0	0.13	0.07
alkane	0	0.13	0.12
Citral	1.03	3.54	0.09
ketone	0.24	0.65	0
benzenediethyldimethyl	0.33	1.22	0.07
alkane	0.08	0.39	0.43
β-Caryophyllene	4.83	5.8	6.03
trans-α-Bergamotene	1.54	1.66	1.91
α-Guaeiene	0.98	0.85	1.44
alkane	0	0.16	0.12
β-Farnesene	2.37	2.56	2.91
Humulene	1.55	1.91	1.97
alcohol	0.13	0.13	0
Longifolene	0.22	0.23	0.2
alkane	0	0.15	0.13
Aromadendrene	0.70	1.21	1.16
Guaiadiene	0.66	0.91	0.8
α-Famesene	0.65	0.83	0.87
β – Bisabolene	0.69	0.46	0.4
2-*epi*-α-Furebrene	0	0.23	0
alkane	0	0.54	0.55
ketone	0.44	0.34	0
m-Anicic acid	0	0.14	0.14
Fumaric acid	0	0.27	0.47
Nerolidol	0.42	0.46	0.16
Neointermedeol	0.35	0.5	0.24
Selinadiene	0.14	0.19	0.13
Bisabolol	0.34	0.48	0.31
alkane	0	0.28	0.24
ketone	0.06	0.33	0
Caryophyllene oxide	0	0.6	0
Valerenadiene	0	0.16	0.08
Cannabidiol (CBD)	0.1	0.14	0.15
Cannabichromene (CBC)	0.76	0.75	0.42
Cannabigerol (CBG)	13.4	12.24	1.12
Cannabinol (CBN)	0.05	0.17	0
Dronabinol (THC)	25.57	30.41	37.8

From week 7 on, the plants were exposed to a flowering light cycle of 12 h of Na-600 W lighting for at least 6 weeks, until harvest. Inflorescence was determined as mature once 70–80% of the pistils turned brown. The inflorescences were sampled at three different locations along the flowering stem, as illustrated in Fig. 1. The analyses were performed within 2 weeks of sampling, in three different sampling

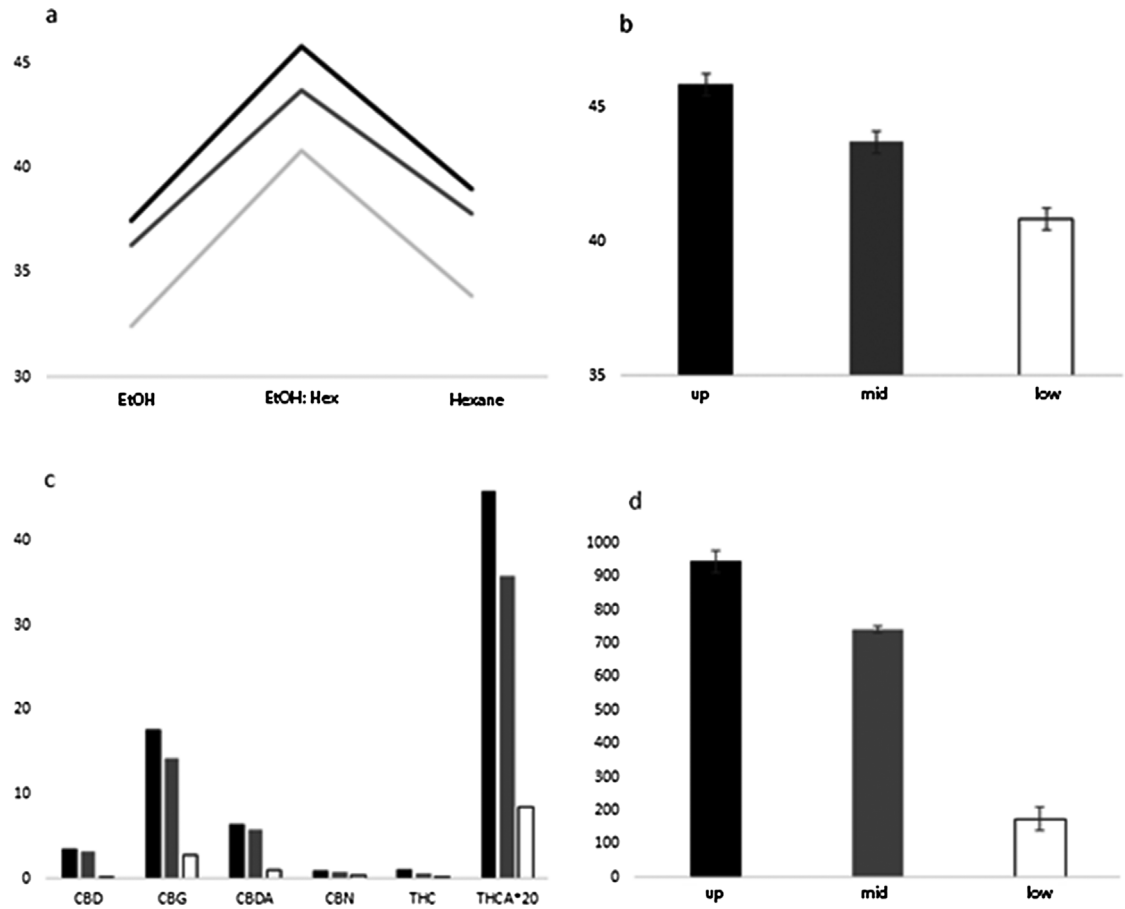

Fig. 2. Comparison of the different solvents used and their effects on the total and maximum amounts (mg mL^{-1}) of active compounds extracted from different inflorescences along the *C. sativa* stem. (a) Total terpenoids extracted by three different extraction methods. EtOH – polar extraction using ethanol as solvent; Hexane – non-polar extraction using *n*-hexane as solvent; EtOH:Hex – extraction using a mixture of ethanol and hexane, 7:3, v/v. Black line – upper inflorescence, dark gray line – middle inflorescence, light gray line – lower inflorescence. (b) Maximum amounts of terpenoids extracted from high (up), middle (mid) and low inflorescences (see Fig. 1) using ethanolic extraction. (c) Total amounts of different cannabinoids extracted by ethanolic extraction. Black column – upper inflorescence, gray column – middle inflorescence, white column – lower inflorescence. (d) Maximum amounts of cannabinoids extracted from high, mid and low inflorescences using ethanolic extraction.

batches. In each batch, one to two inflorescences were sampled for each height, to obtain 0.5 g of material (inflorescences that were lower on the stem were significantly smaller than those above them). The leaves were cut off with pruning shears to ensure that only flowers were extracted. Values determined for light intensity are based on illuminance measurements by Lux meter.

Three cuttings of standardized mother (strain C2F) plant were grown indoors under controlled conditions (25 ± 2 °C and 50–60% humidity). The solvents used for the extractions and separations—ethanol, *n*-hexane, methanol (all GC–MS EMSURE-grade and HPLC-grade), acetic acid, and water were purchased from Mercury Scientific and Industrial Products Ltd. Organic standards used for cannabinoid and terpenoid quantification and retention-time determination were purchased from Sigma-Aldrich Israel Ltd. (under Merck Millipore).

2.2. Methods

2.2.1. Sample preparation

All glassware, and the mortar and pestle were rinsed with acetone. Method blanks were routinely run with each extraction batch. Fresh flowers (0.6 g fresh weight) were weighed and crushed by mortar and pestle after freezing with liquid nitrogen. The homogenized samples were placed in 15 mL vials. Distilled solvent (6 mL) was added to each sample. Three different solvents were used–ethanol, hexane, and a mixture of hexane and ethanol (7:3, v/v), prepared in duplicates. The samples were shaken for 45 min at 225 rpm in a TU-400 orbital shaker incubator at room temperature. The solvent was then decanted to a clean vial. A 1 mL aliquot of the total extract from each sample was placed in a 2 mL sterile glass vial.

Fresh *cannabis* flowers (1 g) were extracted with 4 mL of ethanol. The extract was divided into four equal samples, each containing 900 µL. One sample was kept undried, the second sampled was dried under a gentle stream of nitrogen, the third sample was dried in a speedvac (Chist Alpha RVC, vacuum pressure 10 millibar) and the fourth sample was dried in a rotary evaporator (IKA HB 10 V, 20–180 C, with heating bath IKA RV 8 V, 5–300 rpm and vertical glassware set RV 10.1). The samples were vaporized to complete dryness. Then, 100 µL of ethanol was added to the dried samples prior to injection into the GC–MS. The samples were injected immediately after preparation.

2.2.2. GC–MS

Analyses were carried out using an Agilent 7890B gas chromatograph coupled to a 5977A mass spectrometer (electron multiplier potential 2 kV, filament current 0.35 mA, electron energy 70 eV, and the spectra were recorded over the range of *m/z* 40–500). An Agilent 7683 autosampler was used for sample introduction. A 1-µL aliquot of each sample was injected into the GC–MS using 1:10 split-ratio injection mode. Helium was used as the carrier gas at a constant flow of 1.1 mL s^{-1}. Isothermal hold at 50 °C was maintained for 2 min, followed by a heating gradient of 6 °C min^{-1} until reaching 300 °C, and the final temperature was held for 4 min. A 3-min solvent delay was

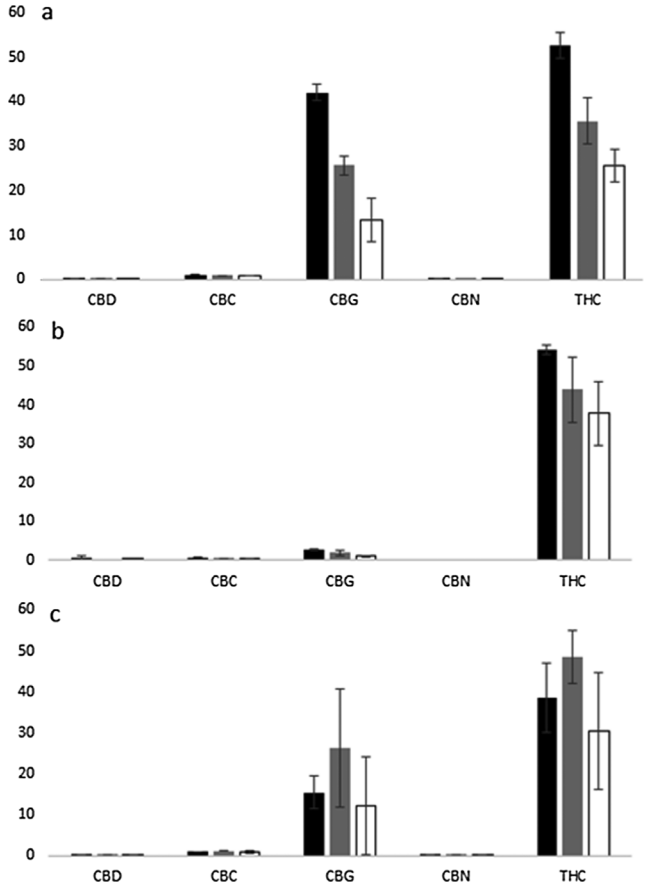

Fig. 3. Comparison of different solvents used and their effects on the amounts (mg mL^{-1}) of cannabinoids extracted from different inflorescences along the *C. sativa* stem. (a) Ethanolic extraction. (b) *n*-Hexane extraction. (c) Mixed solvent extraction (mixture of hexane and ethanol, 7:3, v:v). Black column – upper inflorescence, gray column – middle inflorescence, white column – lower inflorescence.

applied. A 30 m, 0.25 mm ID, 5% cross-linked phenylmethyl siloxane capillary column (HP-5MS) with 0.25-μm film thickness was used for separation and the injection port temperature was 220 °C. The MS interface temperature was 280 °C. Peak assignments were carried out with the aid of library spectra (NIST 14.0) and compared with published data and MS data obtained from the injection of standards purchased from Sigma-Aldrich (Restek).

2.2.3. HPLC

Sample separation was carried out using a Varian Prostar HPLC system coupled with a Varian 410 autosampler, 210 pump, and 320 UV/Vis detector. The separation was performed on a Purospher RP-18 endcapped column (250 mm × 4.6 mm ID; Merck KGaA, Darmstadt, Germany) with a guard column (4 mm × 4 mm ID). Solvent gradients were formed by isocratic proportion with 15% of 0.1% acetic acid in HPLC grade water and 85% HPLC grade methanol at a flow rate of 1.5 mL min^{-1} for 35 min. A 50-μL aliquot of sample was injected. The compound peaks were detected at 220 nm and 280 nm.

2.2.4. Quantification

For GC–MS, 1 μL of each 1-mL sample was injected into the GC–MS using 1:10 split-ratio injection mode. Different terpenes (rose oxide and eucalyptol) and cannabinoids (CBG, CBD, cannabinol [CBN], THC and cannabichromene [CBC]) were used as external standards for yield assessment. Terpene and cannabinoid standards were purchased from Sigma-Aldrich (purity higher than 95%).

For HPLC, the compounds were identified and calibrated against standards of CBG, CBD, cannabidiolic acid (CBDA), CBN, cannabigerolic acid (CBGA), THC, CBC and dronabinol acid (THCA). The cannabinoid standards were diluted to 10 ppm with methanol and then subjected to HPLC analysis as with the samples. THC and THCA were used as external calibration standards for quantification of neutral and acid cannabinoids, at suitable concentrations between 5 ppm and 40 ppm.

3. Results and discussion

Inflorescences sampled along the *C. sativa* flowering stem at three different locations (Fig. 1) were extracted separately. The compounds extracted using polar and non-polar solvents are listed in Table 1, based on their detection and identification using gas chromatography–mass spectrometry (GC–MS). Alkanes were detected by the non-polar extraction method, whereas ketones and alcohols were identified in the polar-based extract. With respect to total lipids extracted, the highest yield (detected by GC–MS and calibrated against an external calibration curve of cannabinoid standards) was achieved when a mixed solution of polar and non-polar solvents was used, regardless of sampling location (Fig. 2a).

The extracted amounts of both cannabinoids and terpenoids were dependent upon the location of the sampled inflorescence along the flowering stem. The highest amount of terpenoids was detected in the uppermost sampled inflorescence, and the lowest amount in the lowermost sampled inflorescence (Fig. 2b). The same relation of amount extracted to inflorescence location was recorded for the cannabinoids present in the extracts, with the highest amount of extracted cannabinoids in the uppermost inflorescence and the lowest in the lowermost inflorescence (Fig. 2c). This decrease moving down the flowering stem was detected independently of the analytical extraction method chosen; i.e., when the same extraction method was compared for all locations, the same decreasing trend was obtained (Fig. 2a).

Comparison of acidic and neutral cannabinoids extraction using organic solvents with different polarity and analyzed by HPLC showed that cannabinoids are best extracted with polar solvents (Fig. 3).

Drying methods using both speedvac and rotary evaporator led to the relatively volatile monoterpenes' degradation to almost non-detectable levels, losing most of the total extracted amount (Fig. 4a). These drying methods also altered the amounts of sesquiterpenes perceived, reducing the gained amount to almost half (Fig. 4b). The lowest damage to terpenoid and cannabinoid compositions was detected when samples were dried under a very gentle stream of nitrogen (Fig. 4c–e). Both monoterpenes (Fig. 4c) and sesquiterpenes (Fig. 4d) remained almost intact. Furthermore, the effects of gas flow and rotary evaporation on the amount of cannabinoids detected were essentially negligible (< 5/100 and 1/10, respectively; Fig. 4e). However, evaporation by speedvac reduced the amounts of THC and cannabigerol (CBG) to two-thirds (Fig. 4e).

Finally, the significant decrease in the amounts of cannabinoids extracted from inflorescences sampled from top to bottom of the *C. sativa* stem was demonstrated (Fig. 2), remained consistent regardless of the detection method used (Fig. 5).

The total amount of cannabinoids, and similarly of terpenoids, extracted using three different solvents showed clear preference to the mixed solution of polar and non-polar organic solvents, regardless of sampling location. Hence, we suggest that the most adequate extraction method for both cannabinoids and terpenoids for yield maximization is a combination of polar and non-polar mixed solvents (Aizpurua-Olaizola et al., 2016).

The extracted amount of total organic compounds showed dependency upon the location of the sampled inflorescence along the flowering stem. The highest amount of organic compounds was detected in the uppermost sampled inflorescence, and the lowest amount in the lowermost sampled inflorescence. This was shown to occur independently of the analytical extraction method chosen. Since it is likely that all flowers along a stem receive the signal for transition to

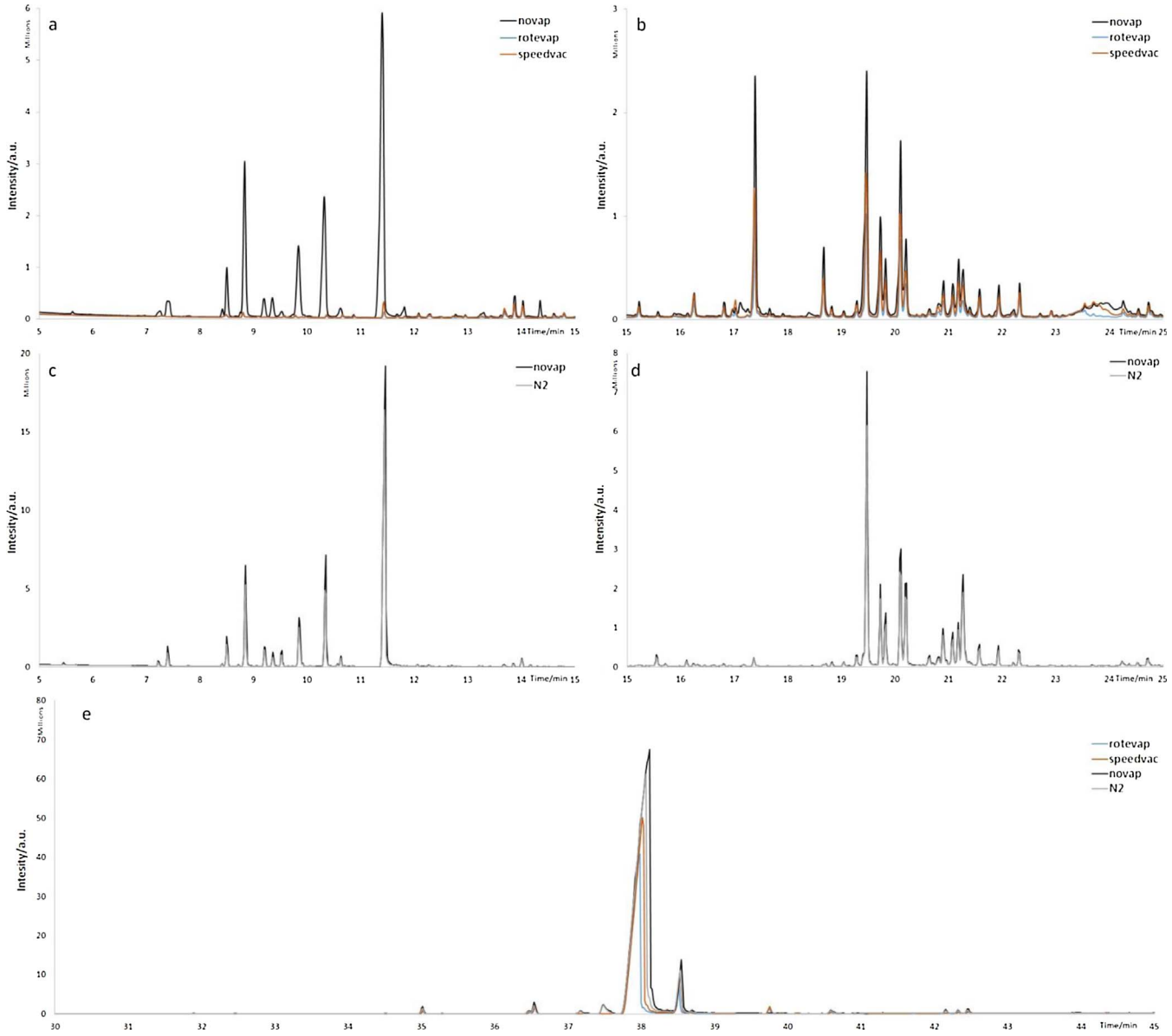

Fig. 4. Effect of rotary evaporation and speedvac drying methods on the amounts of (a) monoterpenes and (b) sesquiterpenes and terpene alcohols. Effect of drying under nitrogen stream on the amounts of (c) monoterpenes and (d) sesquiterpenes and terpene alcohols. Effects of all three drying methods on the amounts of (e) neutral cannabinoids, present in the polar (ethanol) extract and analyzed by GC–MS. Black line – no evaporation used; blue line – rotary evaporation; red line – speedvac evaporation; gray line – drying under a gentle nitrogen stream. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reproductive mode (i.e., exposure to shorter day length; Aizpurua-Olaizola et al., 2016) simultaneously, we suggest that the observed dependence of accumulated phytochemicals on location of the inflorescence is derived from environmental conditions that might affect maturation processes and compound production, once all of the flowers have been simultaneously induced.

Notably, to our understanding, all nutritional and flowering conditions were similar in the current experiments except for distance from the light source, which was different for the various inflorescences flowering along the same flowering stem. As the light source is located above the flowers, and as the stems grow vertically, the upper inflorescences on a stem have more light exposure than inflorescences positioned lower on the same *C. sativa* flowering stem. Moreover, leaves positioned on the flowering stem in close proximity to the inflorescence prevent the light from reaching the lower inflorescences while the upper inflorescences are fully exposed to the light source.

Indeed, measurements of illuminance along a stem showed that the upper inflorescence was exposed to 50,000–45,000 lx, the middle inflorescence to about 20,000–15,000 lx and the low inflorescence to only 7000–5000 lx. This indicates that light exposure for the lower inflorescence is reduced by at least 60% of that reaching the upper inflorescences.

Since, in accordance with the reduction in light exposure, the accumulated amounts of cannabinoids and terpenoids decreased as the sampling location moved down the stem, we suggest that light exposure governs this variance in compounds produced by the plant. Indeed, the level of light exposure may affect the production of the plant compounds. The correlation between light exposure and composition of the chemicals produced by plants has long been recognized (Shread, 1940; Spoehr and Milner, 1939) in a variety of aquatic and terrestrial plants (Loreto et al., 2006; Niinemets, 2010).

Cannabinoids are usually processed and analyzed by HPLC. As cannabinoids are best extracted with polar solvents, they can be directly injected for HPLC without any further treatment. The extracts are routinely dried before application to the analytical separation instrument—GC–MS or HPLC. To evaluate the effect of drying on the

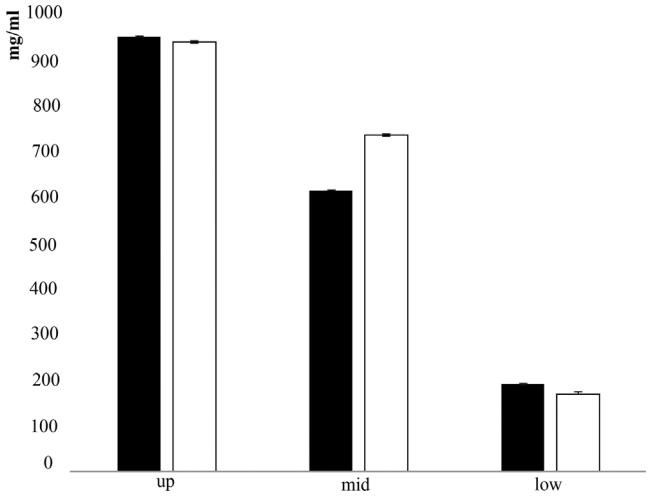

Fig. 5. Total amount of cannabinoids detected by different separation methods (HPLC vs. GC–MS). Results are shown for the ethanolic extracts of inflorescences at the different sampling locations: high (up), middle (mid) and low (see Fig. 1). Black columns – GC separation, white columns – HPLC separation.

amount of cannabinoids and terpenes remaining in the extract, the most common drying methods were compared. The amounts of terpenoids and cannabinoids detected after drying the sample using three different evaporation methods—gentle stream of nitrogen, speedvac or rotary evaporator—were compared with amounts detected with no vaporization treatment.

The effects of three different drying methods, i.e. speedvac, rotary evaporator and drying under a very gentle stream of nitrogen, on the terpenoids and cannabinoids were assessed. Speedvac and rotary evaporator led to the highest loss of the total extracted amount whereas when samples were dried under a very gentle stream of nitrogen the loss of both terpenes and cannabinoids was essentially negligible. Therefore, when possible, it is best to avoid drying of the extracts altogether; other introduction methods that do not require drying, such as headspace or solid-phase micro-extraction (SPME) should be selected (Snyder et al., 1988). When drying is unavoidable, using a gentle stream of nitrogen or any noble gas is preferred. Therefore, the chosen solvents and their initial volume should be designed to fit a minimized evaporation step. Notably, the commonly used overnight speedvac drying should be avoided.

Phytochemical degradation due to extract drying indicates one advantage of HPLC over GC in the analysis of cannabinoids. As the most adequate solvents for the extraction of cannabinoids are those with high polarity, the best-suited separation method is HPLC. Highly polar solvents can be introduced into the HPLC apparatus and thus no evaporation step is required and the original amounts of extracted cannabinoids are retained.

Another advantage of the HPLC over GC–MS is the detection of cannabinolic acids. Like all cannabinolic acids, tetrahydrocannabinolic acid (THCA) degrades to THC as its progresses through the GC injection port. Decarboxylation of cannabinolic acids to their neutral forms occurs only partially under the conditions we applied during GC analysis (see Experimental section for details), and thus not all THCA degrades to THC. This partial decarboxylation process prevents a quantifiable assessment of the extracted amounts of cannabinolic acids. However, during HPLC analysis, cannabinolic acids can be quantified directly and thus reliable assessments can be achieved. Therefore, for both quantification and identification of cannabinoids, HPLC is a more adequate method than GC. Nevertheless, as already noted, regardless of the detection method used, the significant decrease in the amounts of cannabinoids extracted from inflorescences sampled from top to bottom of the *C. sativa* stem remains consistent.

4. Conclusions

We demonstrate the importance of studying technical aspects of *cannabis* growth, extraction and analysis for both qualitative and quantitative levels of cannabinoids and terpenes, and the need for standardization within the methods applied. The fact that upper inflorescences have significantly higher amounts of cannabinoids and terpenoids than those lower down on the stem may have both agricultural and therapeutic consequences. Calibrating the composition and amounts of active compounds produced by the plant is mandatory to increase the effectiveness of patient treatment with *cannabis*. Standardization of the growing processes will lead to reproducible quality and quantity of active phytochemicals and as a result, to the ability to prescribe medical *cannabis* to patients as a regulated medicine. For good agricultural practice, i.e., environmental considerations and lower energy consumption, and for optimal production of active compounds in flowers, careful control of the light source intensity, location and arrangement might be beneficial. Changing the light source position and settings, as well as further pruning the lowermost inflorescences, may have a significant impact on the accumulated amounts of the desired compounds in the remaining *cannabis* flowers.

Moreover, standardizing the method used for *cannabis* extraction is highly important for this plant's medicalization. We showed that polar solvents are best for the extraction of cannabinoids, whereas the most adequate method for a more comprehensive extract of all active compounds is a mixture of polar and non-polar solvents, such as *n*-hexane and ethanol, both permitted for use by the Food and Drug Administration (FDA; Guidance for Industry, 2012). It should be noted that FDA regulations do limit the levels of *n*-hexane in pharmaceutical products (Guidance for Industry, 2012); on the other hand, the somewhat more polar solvent heptane is allowed for use in pharmaceutical products consumed on a daily basis and its use as a solvent to efficiently extract *C. sativa* phytochemicals for therapeutic purposes may be preferred.

We also showed that quantifiable extracts endure only minimal drying; in particular, with monoterpenes, any drying of the extract should be avoided. Another aspect that warrants further investigation is the threshold for positive therapeutic effects. Different extracted amounts may result in variable effectiveness. As different extraction methods result in different amounts of active compounds, the threshold needed for a therapeutic effect may not be reached. Therefore, the method of extraction should be standardized for each *C. sativa*-based product for optimal composition and amount of active compounds. Improved methodology and rational standardization of plant growth and inflorescence extraction may significantly promote *cannabis* medicalization.

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