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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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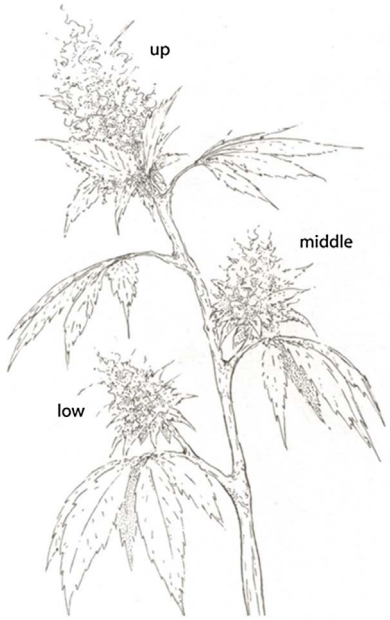

Fig. 1. Graphic illustration of the *Cannabis sativa* flowering stem and positions of the inflorescences. Sampling locations are marked up, middle and low in accordance to their position along the *cannabis* flowering stem (illustrated by Omer Koltai).

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