FLAVOUR AND FRAGRANCE JOURNAL
Flavour Fragr. J. 2001; **16**: 259–262
DOI: 10.1002/ffj.993

Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity

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Received 12 July 2000
Revised 20 December 2000
Accepted 15 January 2001

ABSTRACT: The essential oils of five different cultivars of *Cannabis sativa* contained as main compounds α-pinene, myrcene, *trans*-β-ocimene, α-terpinolene, *trans*-caryophyllene and α-humulene. The content of α-terpinolene divided the cultivars in two distinct groups, an Eastern European group of cultivars of approximately 8% and a French group of cultivars of around 16%. Therefore, this compound might be suitable as a genetic marker for the two breeding centres for the fibre types of *Cannabis sativa*. The content of *trans*-caryophyllene was up to 19%. However, the content of caryophyllene oxide did not exceed 2%. The antimicrobial activity of the essential oil of *Cannabis sativa* can be regarded as modest. Nevertheless, cultivar differences were visible. Δ-9-tetrahydrocannabinol (THC) could not be detected in any of the essential oils and the amount of other cannabinoids was very poor. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: *Cannabis sativa* L.; Cannabaceae; essential oil composition; myrcene; *trans*-β-ocimene; *trans*-caryophyllene; cannabinoids; antimicrobial activity

Introduction

Hemp (*Cannabis sativa* L.) was traditionally cultivated on a large scale in Austria until 1900, when cheaper cotton and other fibre crops began to substitute for hemp fibres. Recently, interest in the production of hemp as an alternative crop has increased[1] due to the wide array of applications from using the bast fibres (textiles, industrial uses, paper and construction), the hurds (paper, construction and agricultural use), leaves (agricultural use) and the seeds (fatty oil in food, as well as industrial uses and whole seeds as animal feed).[2]

A prerequisite to avoid drug abuse of hemp was the successful breeding of cultivars with a content of the hallucinogenous Δ-9-tetrahydrocannabinol (THC) of less than 0.3%. In the EU, cultivation has been regulated since 1970 by guidelines listing cultivars with a content of THC less than 0.3% in the *Common Catalogue of Varieties of Agricultural Plant Species* (88/380/EEC). National regulations allowed national cultivation according to this list. The European Toxicology Experts Working Group defined cut-off values for urine cannabinoids of 50 µl/l for workplace drug testing,[3] a value that could not be reached, even by intensive application of cosmetic products with hemp ingredients.[4]

Cannabis sativa L. also contains an essential oil related in its composition to *Humulus lupulus* L. The composition has already been subject of several investigations.[5,6,7] Hashish detection dogs are trained on the smell of caryophyllene oxide.[8] Furthermore, the essential oil showed bacteriostatic properties.[5] Since, for hemp, agricultural production methods were optimized due to its importance as an industrial crop, competitive production of the essential oil in Europe might be possible.

The aim of this study was to examine the essential oil composition of different cultivars, to check the antimicrobial properties of the essential oils of the different cultivars and to determine the eventual presence of cannabinoids in the essential oil, also by comparing the suitability of different thin-layer chromatographic systems.

Experimental

Source of Essential Oils

An organization of Austrian farmers specialized in production of hemp base material (Hanfbörse-Austria; Amstetten, Austria) provided us with commercially distilled essential oils of five different cultivars (cv. Felina

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34, cv. Fedrina 74, cv. SwissMix, cv. Kompolti and cv. Secuemi).

GC–MS Analysis

The oil (5 µl) was diluted with CH_2Cl_2 (495 µl) prior to analysis. GC–MS-analyses were performed on a HP 6890 coupled with a HP 5972 MSD and fitted with a HP 30 m × 0.25 mm capillary column coated with HP-5MS (0.25 µm film thickness). The analytical conditions were: carrier gas, helium; injector temperature 250 °C; split ratio, 50 : 1, temperature programme, 50 °C to 140 °C at a rate of 5 °C/min and 140 °C to 170 °C at a rate of 2 °C/min. Components were identified by comparing their retention indices [Kováts index (KI)] and mass spectra.[10,11]

TLC Analysis of Cannabinoids

Four different thin-layer-chromatographic systems for the detection of cannabinoids were compared (in the following denominated System I,[12] II,[13] III[13] and IV[14] respectively). Hemp essential oils were diluted (1 : 50) in *n*-hexane; 20 µl of the dilutions were used for TLC.

Reference substances

1. Thymol (Merck, Darmstadt): 10 mg in 20 ml *n*-hexane; 10 µl used for TLC.
2. Colour test mixture: dimethyl yellow, Sudan red, indophenol, each 0.05% in toluene; 10 µl used for TLC.
3. Hashish: 1 : 10 in *n*-hexane; 5 µl used for TLC.
4. Marihuana: 1 g powdered drug was extracted with 10 ml methanol by sonification for 10 min. at room temperature. The filtrate was evaporated and the residue dissolved in 1 ml toluene; 10 µl used for TLC.
   - *Stationary phase: Systems I and II*: HPTLC, Silica gel 60 F254 nm (Merck); *System III*: HPTLC, Silica gel 60 F254 nm (Merck) and impregnation with dimethylformamide/carbon tetrachloride (6 + 4); *System IV*: HPTLC, RP-18, (Merck).
   - *Mobile phase: System II*, *n*-hexane/diethyl ether (8 + 2); *System II*, *n*-hexane/dioxane (9 + 1); development, twice, 5 min. between; *System III*, cyclohexane; development, twice, 10 min. between; *System IV*: acetonitrile/water (9 + 1).
   - *Detection: Fast blue salt reagent–NaOH: (Systems I, II and III)*: 0.5 g fast blue salt B = 3,3′-dimethoxybiphenyl-4,4′-bis(diazonium)-dichloride dissolved in 100 ml water; drying: 10% ethanolic NaOH; inspection in vis. *System IV*: 0.5 g fast blue salt B dissolved in 10 ml water and 90 ml acetone added.
   - *Detection limits*: detection of Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) using fast blue salt reagent, NaOH: 0.01 µg.[15]

Antimicrobial Analysis

Bacterial cultures were grown overnight in IsoSensitest broth prior to use. 0.5 ml volumes of culture were added to 20 ml IsoSensitest agar in Petri dishes and allowed to solidify. Wells 4 mm in diameter were punched into the agar, to which was added 15 µl test substance. The plates were subsequently incubated in darkness at 25 °C for up to 48 h, after which the diameter of the zone of inhibition was measured. The test was done in duplicate. The different test organisms are listed in Table 2. The choice of organisms was made to give a spectrum of bacterial habitats from human, animal and food sources.

Results and Discussion

Essential Oil

The essential oil of *Cannabis sativa* L. (Table 1) contained, as main compounds, α-pinene, myrcene, *trans*-β-ocimene, α-terpinolene, *trans*-caryophyllene and α-humulene. The main differences between the cultivars could be found in the content of α-terpinolene, which separates the cultivars clearly into two groups: group 1, with SwissMix (7%) and the Eastern European cultivars Kompolti (8.3%) and Secuemi (7.1%); and group 2, with the French varieties Felina 34 (16.4%) and Fedrina 74 (16.6%), respectively. This might be a genetic marker for distinguishing two important gene pools for breeding low-THC varieties but should be subject of a more detailed study. Another notable difference was found in the high content of α-pinene in cv. Secuemi (14.6%), in contrast to the other cultivars (7.3–8.8% only).

The essential oil is different to the analysis of Hendriks *et al.*,[5] who found mainly sesquiterpenes (e.g. β-caryophyllene at 37% and β-humulene at 9.4%) and monoterpenes only below 1.5%. Our results differ also strongly from Ross *et al.*,[6] who found myrcene at 67% (highest value in our study 35% at cv. SwissMix) and limonene at 16% compared to 3.5% in cv. Secuemi. The results here are in a general concordance with those of Mediavilla and Steinemann,[7] who partly used the same cultivars cultivated in Switzerland. However, some main compounds differ by up to 30%. Since breeding has a long tradition in *Cannabis sativa*, the cultivars should be quite homogeneous also regarding the essential oils. In this case, the differences can only be attributed to the different regions of cultivation (i.e. different environments) and/or different distillation conditions. Compared to the

Table 1. Essential oil composition of five different cultivars of *Cannabis sativa* L.

Peak #	RI	Compound	ID	SwissMix (%)	Felina 34 (%)	Fedrina 74 (%)	Kompolti (%)	Secuemi (%)
1	931	α-Thujene	RI,MS	0.23	0.21	0.19	0.10	0.23
2	938	α-Pinene	RI,MS	7.25	7.21	8.76	7.30	14.61
3	954	Camphene	RI,MS	tr.	tr.	0.15	0.07	0.24
4	981	β-Pinene	RI,MS	3.13	3.01	3.75	3.19	5.44
5	993	Myrcene	RI,MS	35.02	24.13	29.19	32.62	21.08
6	1007	α-Phellandrene	RI,MS	0.32	0.72	0.77	0.36	0.34
7	1013	Δ-3-Carene	RI,MS	1.80	0.66	0.79	1.09	2.01
8	1020	α-Terpinene	RI,MS	0.13	0.54	0.56	0.26	0.27
9	1034	Limonene	RI,MS	3.02	1.97	2.80	2.98	3.53
10	1036	1,8-Cineol	RI,MS	0.42	0.16	0.17	0.34	0.40
11	1041	*cis*-β-Ocimene	RI,MS	0.64	0.84	0.79	0.88	0.96
12	1052	*trans*-β-Ocimene	RI,MS	9.03	7.33	7.85	9.04	7.48
13	1063	γ-Terpinene	RI,MS	0.11	0.42	0.43	0.23	0.25
14	1092	α-Terpinolene	RI,MS	7.02	16.42	16.61	8.28	7.05
15	1181	Terpinen-4-ol	RI,MS	tr.	0.35	0.30	tr.	tr.
16	1193	α-Terpineol	RI,MS	0.36	tr.	tr.	0.47	tr.
17	1411	*cis*-Caryophyllene	RI,MS	0.12	0.14	0.22	0.32	0.39
18	1418	*cis*-α-Bergamotene	RI,MS	tr.	0.27	0.21	0.12	tr.
19	1425	*trans*-Caryophyllene	RI,MS	16.33	16.52	12.19	16.53	18.93
20	1438	*trans*-α-Bergamotene	RI,MS	1.26	1.93	1.48	0.73	1.00
21	1457	α-Humulene	RI,MS	6.97	8.71	6.10	7.10	7.03
22	1465	*allo*-Aromadendrene	RI,MS	tr.	0.29	0.30	0.27	0.18
23	1487	β-Selinene	RI,MS	tr.	0.29	0.23	0.23	0.18
24	1490	*cis*-β-Guaiene	RI,MS	0.64	0.95	0.79	0.54	0.88
25	1499	α-Selinene	RI,MS	0.66	0.95	0.76	0.59	1.39
26	1509	β-Bisabolene	RI,MS	1.13	0.74	0.47	0.82	0.98
27	1525	β-Sesquiphellandrene	RI,MS	tr.	0.43	0.26	0.11	0.15
28	1560	Germacrene B	RI,MS	0.75	0.38	0.14	0.40	0.49
29	1587	Caryophyllene oxide	RI,MS	1.20	1.58	1.56	1.47	1.33
30	1687	*epi*-α-Bisabolol	RI,MS	tr.	tr.	tr.	tr.	0.16

tr. = <0.1%.

essential oils of two different populations of *Cannabis sativa* subsp. *spontanea*, growing spontaneously in Eastern Austria (Novak J and Franz CM, submitted for publication), the essential oils of the present study showed far lower contents of caryophellene oxide (max. 1.5% in cv. Kompolti vs. 8.4% in an Austrian population).

Antimicrobial Activity

On the test organisms used (Table 2), the essential oil showed antimicrobial activity only in the organisms listed in Table 3. The activity in these organisms can be regarded as modest, compared to different essential oils tested by Dorman and Deans.[16] Nevertheless, cultivar differences of hemp are visible regarding their antimicrobial activity.

Table 2. Organisms tested in the antimicrobial assay

1. *Acinetobacter calcoaceticus*
2. *Aeromonas hydrophyla*
3. *Alcaligenes faecalis*
4. *Bacillus subtilis*
5. *Beneckea natriegens*
6. *Brevibacterium linens*
7. *Brochothrix thermosphacta*
8. *Citrobacter freundii*
9. *Enterobacter aerogenes*
10. *Erwinia carotovora*
11. *Escherichia coli*
12. *Flavobacterium suaveolens*
13. *Klebsiella pneumoniae*
14. *Micrococcus luteus*
15. *Moraxella*
16. *Proteus vulgaris*
17. *Salmonella pullorum*
18. *Serratia marcescens*
19. *Staphylococcus aureus*
20. *Streptococcus faecalis*
21. *Yersinia enterocolitica*

THC Content

Four different TLC systems for analysing cannabinoids were compared (Systems I[12], II[13], III[13] and IV[14]). The best separation of the hallucinogenous cannabinoid tetrahydrocannabinol (THC, R_f value = 40) and the non-hallucinogenous cannabinoids cannabidiol (CBD, R_f = 47), cannabinol (CBN, R_f = 60) and cannabidiol acid (CBA, R_f = 65) could be achieved using System IV with reversed phase material. Treatment with fast blue salt–NaOH reagent showed intense red-violet to red-orange zones in daylight.

The absence of THC in the hemp essential oils from fibre varieties (low in THC content) was stated in all TLC methods and is in accordance to Mediavilla and Steinemann[7] and Novak and Franz (submitted for publication). The amounts of CBD and CBN were very poor.

Table 3. Antimicrobial activity—zone diameter of inhibition (mm)

Cultivar	1*	2	4	5	6	7	11	12	14	19	21
SwissMix	15.0	0.0	11.0	11.3	11.9	10.8	0.0	0.0	0.0	7.1	0.0
Felina 34	7.3	0.0	6.5	7.7	7.4	5.5	0.0	5.3	0.0	14.4	0.0
Fedrina 74	12.1	9.9	0.0	12.1	16.1	4.2	5.8	0.0	0.0	10.0	0.0
Kompolti	11.2	0.0	5.7	9.0	12.7	7.4	0.0	0.0	5.2	5.2	4.8
Secuemi	5.1	0.0	10.7	7.2	0.0	6.4	0.0	0.0	0.0	9.6	0.0

* For identification of organisms refer to Table 2. Organisms not mentioned above were not influenced by the essential oil.

In hashish and marihuana, THC, CBD, CBN and CBA could easily be detected.

Acknowledgements—We acknowledge the provision of essential oils and the financial support of Hanfboerse-Austria (homepage: *www.hanfboerse.at*; e-mail, office@hanfboerse.at).

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