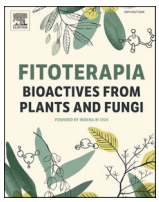
Fitoterapia 178 (2024) 106147

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote



Cannabis sativa essential oils orally administered to CD1 mice: Tissue distribution of main constituents

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ARTICLE INFO

Keywords:
Cannabis sativa
Oral administration
Essential oil
GC–MS

ABSTRACT

The essential oil (EO) obtained from hemp (*Cannabis sativa* L.) biomass is rich of bioactive constituents and its oral administration can be valuable. In this paper two different hemp EOs were orally administered to CD1 mice. One EO, obtained from the fresh plant material, resulted rich in monoterpenes (monoterpene rich oil, MRO) and the other, obtained from the dried biomass, contained mainly sesquiterpenes and CBD (sesquiterpene rich oil, SRO). The blood levels of the most abundant constituents were evaluated in the animals 30 and 90 min after oral administration of hemp EOs. Furthermore, compounds were also measured in brain, liver, kidney, spleen, and cecum content to evaluate their tissue distribution at the same times. Results showed the easy absorption and the ability of the major hemp EOs constituents to reach brain, liver, and kidney. Oral administration of MRO resulted in blood levels of monoterpenes in the range 45–115 ng/g at 30 min and significant tissue distribution with the detection of monoterpenes in brain, liver, and kidney. Oral administration of SRO resulted in blood levels, at 30 min, in the range 70–80 ng/g of sesquiterpenes and 139 ng/g of CBD. The compounds are still detectable in blood and brain 90 min after oral administration and significant concentrations of terpenoids are observed in liver and kidney. MRO and SRO can be considered as valuable sources of these bioactive compounds and further investigations are needed to evaluate the potential uses of hemp EO as constituent of innovative drug formulations.

1. Introduction

Industrial hemp (*Cannabis sativa* L.) is one of the oldest crops cultivated in many areas of the world and different monoecious and dioecious cultivars are grown in various European countries, all of them containing <0.2% of the psychotropic Δ9-tetrahydrocannabinol (THC). Hemp is cultivated to produce edible oil, flour for cooking, fiber for industrial applications, and biomass containing considerable amounts of non-psychotropic cannabinoids, mainly cannabidiolic acid (CBDA), but also a significant amount of volatile terpenes. The latter can be different in relation to cultivar and breeding but mostly are represented by monoterpenes such as α-pinene, myrcene, terpinolene, and bitter-tasting sesquiterpenes α-humulene, (*E*)-caryophyllene, and caryophyllene oxide [8,11]. Much research has been focused on cannabidiol (CBD), which has been included in the first prescription, plant-derived cannabis-based medicine, approved by the US Food and Drug Administration (FDA) and the European Commission (EC) for use in the US and in the EU, respectively. CBD is also under investigation as a treatment for anxiety, pain relief, to improve sleep quality, and many other applications [4–7,12,13,28].

In hemp cultivation, while focusing on producing seeds for food and fibers for various industrial applications, a substantial amount of inflorescences is generated. These inflorescences remain largely underutilized. Currently, they are partially used for extracting CBD and other cannabinoids for cosmetics and pharmaceuticals. However, their full potential remains unexplored. Harnessing these inflorescences could unlock new opportunities, and further research is essential to fully capitalize on these valuable resources.

One opportunity for the valorisation of industrial hemp inflorescences is the extraction of the essential oil (EO) by using distillation techniques, encompassing not only the conventional hydro- and steam distillation, but also novel and most effective and sustainable methods like microwave assisted extraction [16]. Hemp EO has received some attention as a valuable resource that could be exploited in different

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https://doi.org/10.1016/j.fitote.2024.106147
Received 25 March 2024; Received in revised form 23 July 2024; Accepted 28 July 2024
Available online 31 July 2024

fields as a natural product with significant biological properties, including insecticidal, parasiticidal, antimicrobial, and anti-inflammatory [17]. The promising properties of hemp EO can be attributed to its rich chemical profile of monoterpenes and sesquiterpenes, making it an ideal candidate for pharmaceutical, nutraceutical, and agrochemical applications [18]. In this perspective, more studies focused on hemp EO's safety and applicability are needed to solve the actual limits and gaps for its future use as an ingredient in many industrial applications.

In a previous paper, the *in vitro* toxicity of hemp EO was investigated, demonstrating no cytotoxic effects of the product at the tested doses. Additionally, the study revealed its mosquitocidal properties and promising anti-inflammatory activity on human dermal fibroblasts and keratinocytes cell lines [20]. In addition, hemp EOs and their encapsulated forms (nanoemulsions, NEs) proved to possess comparable cytotoxicity on three cell lines used as models for topical and inhalant administration. As a result, the treatment with these products led to lower levels of proinflammatory cytokines compared to etoposide (positive control) without alteration of the basal level of inflammatory cytokines, suggesting the possible safe use of hemp EOs and their NEs for medicinal and therapeutic applications [1].

The sesquiterpene composition of hemp EO mainly consists of (*E*)-caryophyllene, α-humulene, and caryophyllene oxide. Such compounds are claimed to have several bioactivities, and the hemp EO's sesquiterpenes have been considered as responsible for the EOs' anti-inflammatory activity, suggesting that they could serve as promising candidates for the prevention or mitigation of neuroinflammation [3]. (*E*)-Caryophyllene was also approved by FDA for food use as a "dietary cannabinoid", and in general, most of the terpenoids are generally recognised as safe (GRAS) substances and have been included in the EU database on "food flavourings" [21]. A previous paper on (*E*)-caryophyllene revealed its importance as a non psychoactive CB2 receptor ligand and as a significant anti-inflammatory compound. One peculiar aspect of this compound is that it represents an unusual CB2 receptor selective agonist based on a cyclobutene containing chemical scaffold completely different from the other cannabinoids [10]. (*E*)-Caryophyllene and α-humulene were also effective as acaricidal agents against ticks and mites [25].

The hemp EO can be potentially administered sublingually dispersed into drops, by inhalation if diluted into an aerosol, by oral administration when dissolved into a fixed oil, or by skin application if incorporated into creams, ointments, and oils. The oral use of EOs is not common due to the high concentration of natural terpenes, characterized by a pungent and intense odour, and in some cases distasteful taste. Nevertheless, some EOs are normally administered orally, such as the *Lavandula angustifolia* Mill. EO that is also included in a traditional herbal medicine in Europe [15]. Also, *Pimpinella anisum* L. EO can be employed as a flavouring agent in beverages, candies, bread, and cakes, as an additive to inhibit food degradation [22]. EOs, as a main group of phytogenic feed additives, are regarded as safe and cost-effective alternatives to antibiotics in animal diets and as growth promotors. However, some limitations for EOs as feed additives can be identified, including their poorly understood mechanism of action, especially in *in vivo* conditions [23]. In a previous paper, the *in vivo* distribution of the sesquiterpenes present in the EO of *Aegle marmelos* (L.) Corr. was studied in mice, evaluating the levels in the blood and in different tissues. (*E*)-caryophyllene, α-humulene, γ-muurolene, and *ar*-curcumene tissue levels were measured at 30 and 60 min after oral administration [19].

The aim of this work was to study the tissue distribution of the main constituents of hemp EO after oral administration to CD1 mice. The work deals with the measure of the main bioactive monoterpenes, sesquiterpenoids, and CBD contained in the hemp EO, in the animals' blood and tissues. This will offer the opportunity to evaluate the hemp EO as a valuable source of bioactive compounds and to investigate its absorption and distribution after oral administration.

For this study, we orally administered two different hemp EOs to mice. One EO obtained from the fresh plant material and indicated as monoterpene-rich essential oil (MRO), had almost no detectable CBD but was rich in the most volatile fraction, namely monoterpenes. The other EO obtained from the dried biomass, was named sesquiterpene-rich essential oil (SRO), and contained CBD and other volatile compounds, mainly represented by sesquiterpenes.

2. Materials and methods

2.1. Plant biomass

The female inflorescences of Carmagnola CS hemp variety were cultivated and harvested in August 2019 at La Biologica Società Cooperativa Agricola, located in Fiuminata (central Italy, 43°10′40″ N, 12°56′59″ E, 451 m a.s.l.). The Herbarium specimen was identified by one us (F. Maggi) and deposited at the Herbarium of the Centro Ricerche Florisitche dell'Appennino (APP), Barisciano, L'Aquila, Italy, under the code APP 60530. A portion of the plant material was immediately sent to the University of Camerino to be processed as fresh, while another hemp sample was dried at room temperature in the dark before being subjected to distillation.

2.2. EOs extraction

A sample of 2500 g of fresh hemp inflorescences was processed through steam distillation in an E0106 stainless steel apparatus of 20 L capacity by Albrigi Luigi (Stallavena di Grezzana-Verona, Italy) for 3 h. Two L of distilled water were employed and placed at the bottom of the reactor for the distillation process to obtain MRO. On the other hand, hydrodistillation for 3 h was performed for dry hemp inflorescences (100 g) with 3 L of distilled water in a 6 L capacity glass flask equipped with a mantle system Falc MA (Falc Instruments, Treviglio, Italy) and a Clevenger-type apparatus, providing SRO. The obtained EOs were collected into PTFE-silicon septa-sealed amber vials and stored at +4 °C until use. In both cases, the yield estimation was made on a dry weight basis as g of EO/100 g of plant material.

2.3. EOs GC–MS analysis

The chemical profile of the two EOs (MRO and SRO) was assessed by Gas Chromatography coupled to Mass Spectrometry (GC–MS) analysis. The employed equipment consisted of an Agilent 6890 N gas chromatograph with a single quadrupole 5973 N mass spectrometer and an auto-sampler 7863 (Agilent, Wilmington, DE). The capillary column used for compounds separation was a HP-5 MS (5% phenylmethylpolysiloxane, 30 m l., 0.25 mm i.d., 0.1 μm f.t. by J & W Scientific, Folsom). The temperature ramp of the instrument oven was the following: 60 °C for 5 min, then 4 °C/min up to 220 °C, then 11 °C/min up to 280 °C, kept for 15 min. The temperature of the injector and detector was 280 °C, and He (99%) was used as carrier gas at a flow rate of 1 mL/min. The used split ratio was 1:50, and the range for mass acquisition was 29–400 *m/z*, working by the electron-impact (EI, 70 eV) mode. The EOs were diluted 1:100 in *n*-hexane, and 2 μL of each solution were injected. Data processing was carried out through the MSD ChemStation software (Agilent, Version G1701DA D.01.00) and the NIST Mass Spectral Search Program for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library v. 2.3. For peak assignment, analytical standards from Sigma-Aldrich (Milan, Italy) were used, along with the correlation of retention indices [29] and mass spectra with respect to those belonging to ADAMS, NIST 17, and FFNSC2 libraries ([30]; NIST 17, 2008; FFNSC 2, 2012). Peak area normalization without response factors was conducted for the semi-quantification of essential oils constituents.

2.4. In vivo studies

Animals CD1 mice were bred and housed in the animal facility of the University of Padova. Animals were housed in polycarbonate cage under conditions of optimum light, temperature and humidity (12:12 h light–dark cycle, 22 ± 1 °C, 50–60% humidity) with food and water provided *ad libitum*. Experiments were performed according to the Italian Law 26/2014 and European directive 2010/63/UE. Experimental protocols were re-viewed and approved by the Institutional Animal Care and Use Committee (OPBA) of the University of Padova, Italy, and approved by the Italian Ministry of Health 489/2019PR.

Ten weeks old, non-fasted, female mice were randomly placed in the different experimental groups ($n = 6$ each). Each animal received a dose of 20 mg/kg EO diluted in olive oil (100 μL) by oral gavage (20 μL) with a button needle connected to an insulin syringe. The animals were sacrificed after 30 or 90 min from oil administration, and blood, brain, liver, kidney, spleen, and cecum content were taken and stored at −80 °C for the following analysis.

2.5. Tissue preparation and analysis of volatile compounds by GC–MS

Tissues were weighed and transferred in Eppendorf; 300–500 mg of kidney, liver, and brain samples were exactly weighted, and 150 μL of blood were used. For blood, an equal amount of anethole solution (50 μg/mL) in methanol was added as internal standard, and the sample was centrifuged. The supernatant was transferred to a vial for analysis. For tissues, the weighted materials were added with 500 μL of a standard solution of anethole (112 μg/mL) in ethyl acetate, and tissues were chopped. Further 1 mL of ethyl acetate was added, and samples were sonicated for 15 min. Then, samples were centrifuged, and extraction of solid material was repeated. Solutions were collected and gently dried under nitrogen flow, then the residue was dissolved using hexane (100 μL) and used for the GC–MS analysis.

For the calibration curve, a standard solution of anethole (502 μg/mL in diethyl ether) was added in different ratios to a 10 mg/mL solution of hemp EO. Calibration curves were obtained using anethole, and the single quantified constituents and curves are reported in the supplementary materials (exemplicative chromatograms S1 and calibration curves S2-S12). The equations of the calibrations are reported in Table 1.

For the analysis, a Varian 3800 gas chromatograph was used, coupled with Saturn 2000 T MS mass spectrometer using EI as ionization source. For quantitative purposes, the mass spectrometer was set to work in MS/MS mode selecting *m/z* 91 as a precursor for monoterpenes and sesquiterpenoids, while for CBD, the selected ion was the *m/z* 231. The GC–MS/MS method allowed the identification and quantification of the EOs main constituents, leading to measuring their tissue distribution. The injector temperature was 225 °C, the oven started at 55 °C, stayed isothermal for 5.5 min then increased to 250 °C at 4 °C/min and then to 280 °C at 11 °C/min.

Table 1
Calibration curves obtained for the main compounds, along with equation LOD and LOQ.

Compound	Regression	LOD and LOQ
α-pinene	y = 2.16 x − 0.072	1 ng/mL 3 ng/mL
β-pinene	y = 1.98 x − 0.012	1 ng/mL 3 ng/mL
myrcene	y = 3.078 x − 0.011	1 ng/mL 3 ng/mL
limonene	y = 1.33 x − 0.025	1 ng/mL 3 ng/mL
(*E*)-β-ocimene	y = 4.173 x − 0.016	1 ng/mL 3 ng/mL
terpinolene	y = 4.022 x − 0.031	1 ng/mL 3 ng/mL
(*E*)-caryophyllene	y = 0.0288 x − 0.0001	1 ng/mL 3 ng/mL
α-humulene	y = 0.362 x + 0.001	1 ng/mL 3 ng/mL
allo-aromadendrene	y = 0.6184× - 0.0006	1 ng/mL 3 ng/mL
caryophyllene oxide	y = 0.3664× - 0.0004	1 ng/mL 3 ng/mL
cannabidiol	y = 0.385 x − 0.0005	1 ng/mL 3 ng/mL

3. Results and discussion

3.1. EOs characterization

The detected yields were 0.17 and 0.11% *w*/w for the two EOs from dry (SRO) and fresh (MRO) hemp inflorescences, respectively. The total amount of identified compounds in the SRO was 90.4%, of which the most abundant classes of constituents were sesquiterpenes hydrocarbons (41.9%) and, to a minor extent, monoterpene hydrocarbons (26.4%) (Table 2). Among the first ones, the predominant component was (*E*)-caryophyllene (22.7%), followed by α-humulene (8.7%), and caryophyllene oxide (6.0%). Within the second category, myrcene (8.8%), α-pinene (4.9%), and terpinolene (4.5%) represented the main components. On the other hand, for the MRO the 99.8% of the chemical profile was identified, mostly consisting of monoterpene hydrocarbons (81.1%), with myrcene (38.6%), terpinolene (17.0%), α-pinene (10.9%), and limonene (5.4%) as the most significant compounds. Secondly, sesquiterpene hydrocarbons accounted for 18.6%, represented only by (*E*)-caryophyllene (14.7%), and α-humulene (3.9%). The chemical profiles of the two EOs resulted to be different also based on the content of oxygenated mono- and sesquiterpenes, and CBD as the main identified cannabinoid, which were detected in SRO, while they were missing in MRO. Such differences in the EOs' composition related to the status of the plant material have been previously highlighted in other papers [8,9], confirming that drying and storage contribute to the loss of more volatile monoterpenes and to the concentration of cannabinoids in the neutral form.

3.2. Tissue distribution of EOs

In this study, the oral administration of hemp EOs was investigated using CD-1 mice. After oral gavage, animals were sacrificed at 30 and 90 min, and blood, as well as principal organs were analysed. The eleven constituents (Chart 1) of the two EOs belonging to monoterpene, sesquiterpene classes as well as CBD were measured in different animal tissues.

The blood levels of monoterpenes and sesquiterpenes after 30 min were in the range of 15–150 ng/g, both for MRO and SRO (Tables 3,4 and Fig. 1). A higher amount of α- and β-pinene, terpinolene, (*E*)-β-ocimene, and limonene was observed in the animals treated with MRO. This result aligns with the higher concentration of these components in MRO, as indicated by the EO composition. For myrcene, the blood levels of the two groups of animals at 30 min were comparable. This suggests a different kinetic of absorption from the two EOs, considering that myrcene accounts for 38% in MRO and only 8.8% in SRO. When considering the sesquiterpenes (*E*)-caryophyllene and α-humulene, blood levels in the animal receiving SRO and MRO are in agreement with the higher amount of these compounds in SRO (Table 3). In the liver comparable levels of (*E*)-caryophyllene are observed for SRO while α-humulene levels in the liver in MRO are half of the one observed in SRO reflecting the blood concentrations. This observation suggests that absorption may be influenced by the presence of other components. Although preliminary, these data hint that the EO composition and the presence of other constituents in the EO itself may influence the pharmacokinetics of single constituents.

Considering the amount of EO constituents found in the different tissues, it can be observed that the brain and kidney contents (Table 4), are comparable in terms of magnitude order, suggesting that, at 30 min after oral administration, there is a sort of equilibrium between circulating levels in the blood and the main organs (brain, kidney, and liver). A general graph showing the amount of the different compounds in the analysed tissues at 30 min is reported (Fig. 1).

The analysis of the brain tissue shows a similar pattern of compounds as observed in the blood, suggesting that the non-polar constituents in the EO can easily penetrate the blood-brain barrier. A similar trend was observed in the kidney. Due to their lipophilic nature and molecular

Table 2
Chemical profiles of the EOs from dry and fresh CS hemp inflorescences.

No.	Component[a]	RI[b]	RI Lit[c]	SRO (%)	MRO (%)	ID[d]
1	2-heptanone	892	889	0.1		RI, MS
2	*n*-heptanal	903	901	0.1		RI, MS
3	5,5-Dimethyl-1-vinylbicyclo [2.1.1]hexane	915	920	0.2		RI, MS
4	tricyclene	917	921		tr[e]	RI, MS
5	α-thujene	922	924	0.1	0.1	RI, MS
6	α-pinene	927	932	4.9	10.9	Std
7	camphene	940	946	0.2	tr	Std
8	benzaldehyde	956	952	tr		RI, MS
9	sabinene	966	969	0.1		Std
10	β-pinene	969	974	2.4	4.0	Std
11	3-octanone	986	979	tr		RI, MS
12	myrcene	990	988	8.8	38.6	Std
13	α-phellandrene	1003	1002	0.2	0.5	RI, MS
14	δ-3-carene	1008	1008	0.2	0.2	RI, MS
15	α-terpinene	1015	1012	0.2	0.3	Std
16	*p*-cymene	1022	1020	0.1		Std
17	limonene	1026	1024	3.2	5.4	Std
18	1,8-cineole	1027	1026	1.0	0.1	Std
19	(*Z*)-β-ocimene	1038	1032	0.1	0.1	Std
20	(*E*)-β-ocimene	1047	1044	0.9	3.7	Std
21	γ-terpinene	1056	1054	0.3	0.2	Std
22	*cis*-sabinene hydrate	1064	1065	0.3		RI, MS
23	terpinolene	1086	1086	4.5	17.0	Std
24	6,7-epoxymyrcene	1095	1090	tr		RI, MS
25	*trans*-sabinene hydrate	1096	1098	0.2		RI, MS
26	linalool	1101	1095	0.7		Std
27	*endo*-fenchol	1110	1114	0.6		RI, MS
28	*trans*-pinene hydrate	1117	1119	0.4		RI, MS
29	*cis*-menth-2-en-1-ol	1119	1118	0.1		RI, MS
30	*allo*-ocimene	1129	1128	tr		RI, MS
31	*trans*-pinocarveol	1134	1135	0.1		RI, MS
32	*cis*-pinene hydrate	1137	1139	0.1		RI, MS
33	camphene hydrate	1142	1145	tr		RI, MS
34	epoxy-terpinolene	1144	1142	0.2		RI, MS
35	ipsdienol	1147	1140	0.1		RI, MS
36	borneol	1161	1165	0.2		Std
37	terpinen-4-ol	1173	1174	0.8		RI, MS
38	*p*-cymen-8-ol	1184	1179	0.1		RI, MS
39	α-terpineol	1187	1186	0.8		Std
40	*cis*-piperitol	1192	1195	tr		RI, MS
41	*trans*-piperitol	1205	1207	0.1		RI, MS
42	*trans*-chrysanthenyl acetate	1222	1235	tr		RI, MS
43	citronellol	1232	1223	tr		RI, MS
44	eugenol	1356	1356	tr		RI, MS
45	α-ylangene	1364	1373	0.1		RI, MS
46	α-copaene	1368	1374	tr		RI, MS
47	hexyl hexanoate	1389	1382	tr		RI, MS
48	(*Z*)-caryophyllene	1398	1408	0.5		RI, MS
49	α-*cis*-bergamotene	1404	1411	tr		RI, MS
50	(*E*)-caryophyllene	1411	1417	22.7	14.7	Std
51	α-*trans*-bergamotene	1431	1432	0.6		RI, MS
52	6,9-guaiadiene	1437	1442	0.1		RI, MS
53	α-humulene	1444	1452	8.7	3.9	Std
54	allo-aromadendrene	1451	1458	0.6		RI, MS
55	geranyl acetone	1453	1453	0.1		RI, MS
56	(*E*)-β-farnesene	1457	1454	0.3		Std
57	β-chamigrene	1468	1476	0.2		RI, MS
58	γ-muurolene	1470	1478	0.1		RI, MS
59	β-selinene	1476	1489	2.0		RI, MS
60	valencene	1484	1496	0.4		RI, MS
61	α-selinene	1486	1498	1.7		RI, MS
62	α-zingiberene	1495	1493	0.1		RI, MS
63	α-bulnesene	1498	1509	0.4		RI, MS
64	δ-amorphene	1500	1511	0.2		RI, MS
65	β-bisabolene	1505	1505	0.2		RI, MS
66	(*E,E*)-α-farnesene	1508	1505	0.7		RI, MS
67	δ-cadinene	1517	1522	0.1		RI, MS
68	β-sesquiphellandrene	1519	1522	0.1		RI, MS
69	selina-4(15),7(11)-diene	1525	1544	0.4		RI, MS
70	selina-3,7(11)-diene	1531	1538	1.8		RI, MS
71	α-calacorene	1535	1544	0.1		RI, MS
72	(*E*)-neridol	1563	1561	0.4		RI, MS
73	caryophyllene-oxide	1572	1582	6.0		Std
74	humulene epoxide II	1598	1608	1.9		RI, MS
75	caryophylla-4(12),8(13)-dienol-5-ol	1626	1639	0.9		RI, MS
76	α-bisabolol	1678	1685	0.3		RI, MS
77	eudesm-7(11)-en-4-ol	1684	1700	0.3		RI, MS
78	hexahydrofarnesyl acetone	1845	1846	0.2		RI, MS
79	cannabidivarol	2209	2208	0.1		RI, MS
80	cannabicitran	2260	2261	tr		RI, MS
81	cannabidiol	2420	2430	5.3		Std
82	cannabichromene	2439	2440	0.1		RI, MS
83	Δ-9-tetrahydrocannabinol	2534	2529	0.3		RI, MS
84	heptacosane	2700	2700	tr		RI, MS

(*continued on next page*)

Table 2 (*continued*)

No.	Component[a]	RI[b]	RI Lit[c]	SRO (%)	MRO (%)	ID[d]
85	nonacosane	2900	2900	tr		RI, MS
	Total identified (%)			90.4	99.8	
	Grouped compounds (%)					
	Monoterpene hydrocarbons			26.4	81.1	
	Oxygenated monoterpenes			6.1	0.1	
	Sesquiterpene hydrocarbons			41.9	18.6	
	Oxygenated sesquiterpenes			9.8		
	Cannabinoids			5.8		
	Others			0.4		

[a] Order of elution by an HP-5MS column (30 m × 0.25 mm, 0.1 μm).
[b] Linear retention index according to Van den Dool and Kratz [29].
[c] RI from ADAMS and/or NIST 17 and FFNSC3 libraries.
[d] Identification method: Std, comparison with analytical standard; RI, coherence of the calculated RI with those found in ADAMS, NIST 17, and FFNSC3 libraries; MS, mass spectrum overlapping with those reported in ADAMS, NIST 17, WILEY 275, and FFNSC3 libraries.
[e] tr, compounds level < 0.1%.

weight, all the main compounds are highly absorbed, and significant amounts can be observed in both blood and tissues. Surprisingly, CBD is the most abundant compound in the blood of animals treated with SRO, even though is not the most abundant compound in the EO. In the liver, compounds were also detectable, with CBD present to a minor extent. It is possible that in the liver, the compounds undergo phase 2 metabolism, producing non-volatile derivatives such as CBD-glucuronide or CBD-sulphate. As expected, compounds were not detectable in cecum content (data not shown), since after 30 min from oral administration, the bolus did not reach this anatomical site.

The data obtained at 30 min clearly show that the absorption of the main compounds is efficient from both MRO and SRO, and the compounds easily reach the brain, kidney, and liver.

Considering the detectable compounds 90 min after the oral ingestion, we recorded significant changes and data are summarised in Tables 5 and 6. Blood levels were strongly reduced compared to 30 min, ranging from 0 to 3.5 ng/g (Table 5). Nevertheless, CBD, α-humulene, (*E*)-β-ocimene, and (*E*)-caryophyllene were still observed at higher concentrations in the blood of treated animals. In the MRO treated animals at 90 min (*E*)-caryophyllene, α-humulene, (*E*)-β-ocimene, limonene, and myrcene were detected in the blood. However, the more volatile monoterpenes such as α- and β-pinene were not detectable at 90 min in either group of animals, suggesting a rapid elimination of these constituents.

In the liver, significant amounts of the various EO constituents, in the range of 2–20 ng/g, were detected at 90 min (Table 5). Interestingly, the more volatile monoterpenes, α- and β-pinene, were observed, although they were not detectable in the blood at 90 min. In the case of SRO, we observed that limonene in liver at 90 min was present in higher levels compared to CBD and (*E*)-caryophyllene. This may suggest that the more volatile constituents can undergo different distribution routes, and their pharmacokinetics need to be carefully investigated.

The levels of the main EO constituents in the brain appear to be in line with the measured blood levels, except for CBD, which appears to be more concentrated in the brain, suggesting an accumulation effect (Table 6). Comparing the brain, liver, and blood levels, we can argue that the different amounts observed at 90 min may be related to the more efficient phase 2 metabolism at the level of the liver and blood compared to the brain.

In our measurements of kidney levels at 90 min, EO constituents still showed significantly high levels, comparable to the 30-min measurements. Therefore, we can postulate that such compounds exhibit significant tropism for the kidney when administered orally as EO.

The cecum content was also analysed, and high levels of EO

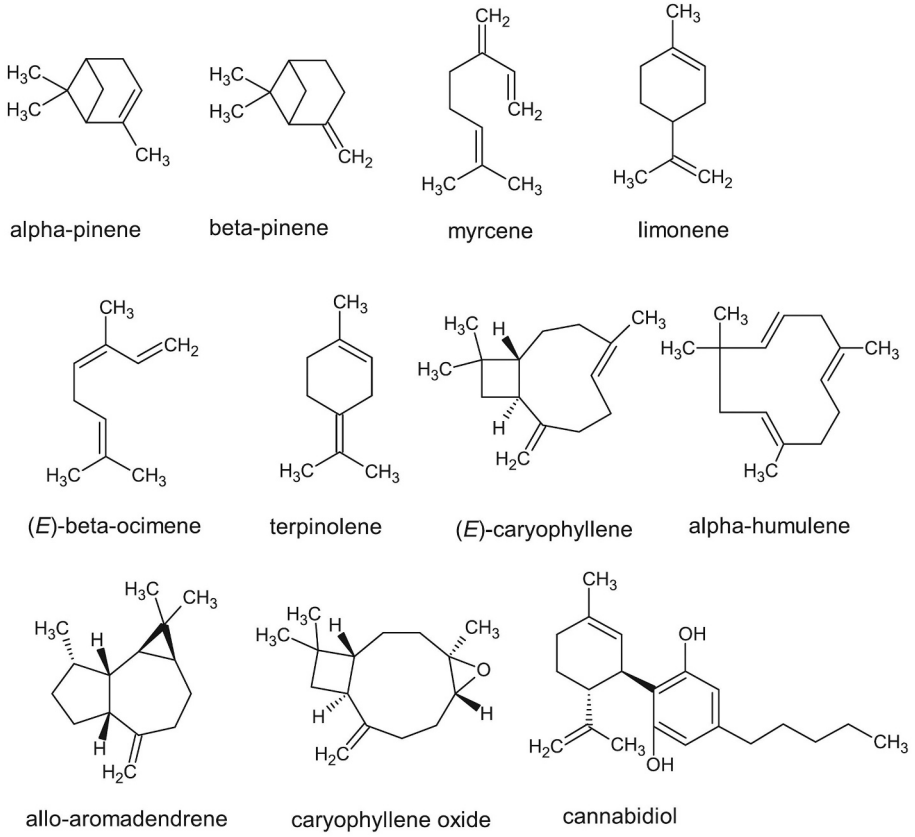

Chart 1. The constituents of the two EOs detected and quantified in blood and tissues after oral administration of EOs.

Table 3
Levels of main constituents of MRO and SRO in blood, and liver 30 min after oral administration.

Compound	Blood MRO 30 min ng/g	DVstd	Blood SRO 30 min ng/g	DVstd	Liver MRO 30 min ng/g	Dv std	Liver SRO 30 min ng/g	Dv std
α-pinene	78,83	12,84	38,46	20,16	127,24	79,74	92,89	64,00
β-pinene	47,65	4,49	20,61	10,85	88,00	5,86	71,76	25,28
myrcene	80,62	53,50	87,12	46,16	106,98	19,61	50,49	23,00
limonene	45,50	35,00	25,50	37,00	22,85	12,00	21,50	15,50
(*E*)-β-ocimene	64,76	29,66	43,19	43,95	38,47	10,80	34,80	5,30
terpinolene	114,66	70,28	91,05	60,00	91,91	32,00	50,67	12,00
(*E*)-caryophyllene	17,90	13,00	69,40	9,00	114,83	13,00	123,38	9,00
α-humulene	45,04	25,00	80,71	8,00	17,83	15,00	39,90	8,00
cannabidiol			138,36	13,00			42,19	13,00

Table 4
Levels of main constituents of MRO and SRO in brain, and kidney 30 min after oral administration.

Compound	Brain MRO 30 min ng/g	Dv Std	Brain SRO 30 min ng/g	Dv Std	Kidney MRO 30 min ng/g	Dv Std	Kidney SRO 30 min ng/g	Dv Std
α-pinene	64,54	24,85	43,70	12,00	84,37	15,00	30,00	17,20
β-pinene	56,52	25,41	83,39	25,28	48,30	18,08	41,20	11,33
myrcene	52,27	23,00	48,28	11,00	38,22	23,00	88,58	32,00
limonene	35,80	13,24	20,80	9,00	18,80	12,00	7,10	2,00
(*E*)-β-ocimene	9,93	6,13	20,52	12,00	46,97	25,00	33,24	16,00
terpinolene	19,14	9,00	9,48	3,50	38,02	12,00	96,96	26,83
(*E*)-caryophyllene	9,48	13,00	50,67	5,00	17,80	13,00	59,18	9,00
α-humulene	35,80	5,00	43,39	8,00	53,27	16,00	63,01	8,00
cannabidiol			96,53	3,50			42,55	12,00

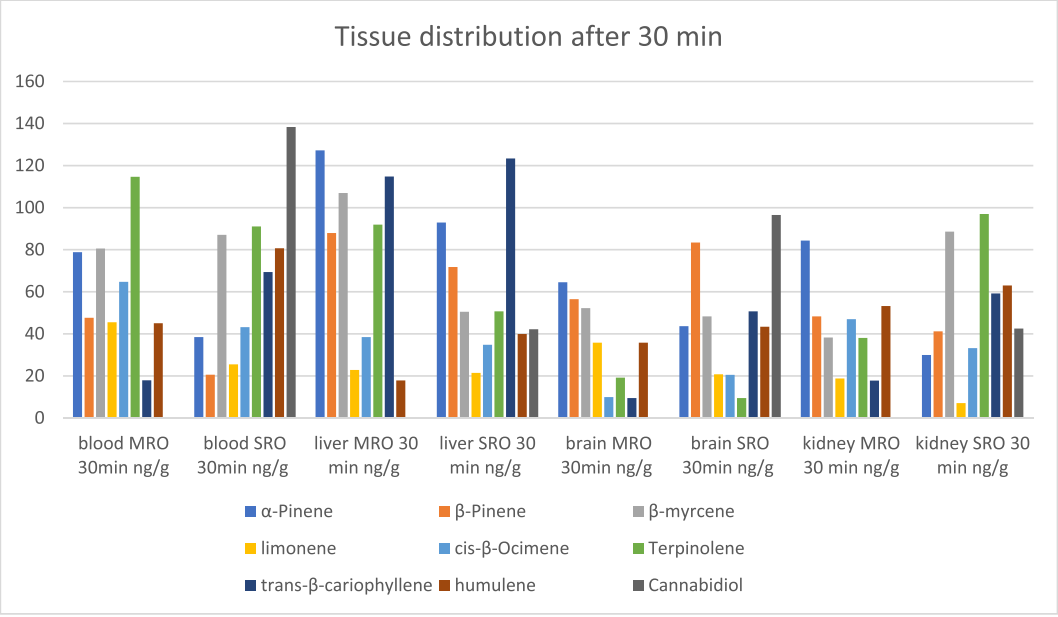

Fig. 1. Levels of analysed volatile compounds from SRO and MRO in blood and tissues of animals after 30 min from oral administration.

Table 5
Levels of main constituents of MRO and SRO in blood and liver 90 min after oral administration.

Compound	Blood MRO 90 min ng/g	Dv Std	Blood SRO 90 min ng/g	Dv Std	Liver MRO 90 min ng/g	Dv Std	Liver SRO 90 min ng/g	Dv Std
α-pinene					18,14	8,00		0,00
β-pinene					8,33	4,10		0,00
myrcene	0,99	0,40	0,21	0,10	3,48	1,60	3,32	1,00
limonene	0,85	0,60	0,15	0,10	9,73	4,60	9,23	4,00
(*E*)-β-ocimene	1,66	0,80	0,21	0,10	3,28	1,10	2,39	1,10
terpinolene					4,57	2,65	4,07	1,60
(*E*)-caryophyllene	2,07	1,00	3,29	0,50	1,20	0,65	1,18	0,36
α-humulene	1,30	0,80	1,21	0,60	1,09	0,60	1,63	0,80
cannabidiol			3,28	0,55			1,24	0,82

Table 6
Levels of main constituents of MRO and SRO in brain and kidney 90 min after oral administration.

Compound	Brain MRO 90 min ng/g	Dv Std	Brain SRO 90 min ng/g	Dv Std	Kidney MRO 90 min ng/g	Dv Std	Kidney SRO 90 min ng/g	Dv Std
α-pinene					15,00	6,50	17,20	6,00
β-pinene					18,08	8,00	11,33	6,00
myrcene	1,02	0,40	0,33	1,00	23,00	10,00	32,00	15,00
limonene	0,99	0,60	0,35	4,00	12,00	5,00	2,00	0,60
(*E*)-β-ocimene	0,96	0,80	0,15	1,10	25,00	15,00	16,00	9,00
terpinolene				1,60	12,00	6,00	26,83	11,00
(*E*)-caryophyllene	0,78	1,00	3,59	0,36	13,00	4,00	9,00	5,00
α-humulene	0,64	0,80	1,60	0,80	16,00	9,00	8,00	3,00
cannabidiol			18,52	0,82			12,00	4,00

constituents were detected, being one order of magnitude higher compared to other districts (Table 7), suggesting that part of the orally administered material remains not absorbed in the lumen of the gut. Furthermore, the detection of a high amount of (*E*)-caryophyllene may suggest that this compound, as well as α-humulene, may be involved in enterohepatic circulation. In particular, high levels of (*E*)-caryophyllene, terpinolene, and CBD were detected.

A general overview of the tissue distribution is represented in the Fig. 2 and at 90 min a large amount of constituents is detected in kidney while blood and brain levels appear to be limited.

4. Discussion

The results of this preliminary study on tissue distribution of hemp EO constituents reveal that the oral administration of hemp EO can be an effective method for delivering bioactive sesquiterpenoids and monoterpenoids, as well as CBD to different tissues. The comparison of the results obtained administering MRO and SRO offers the opportunity to explore the absorption and tissue distribution of bioactive compouds from EO with two different composition. For both MRO and SRO the measurements at 30 and 90 min show the presence of secondary metabolites in all the different considered tissues. Although preliminary, our data suggest that the administration of EO with different composition may result in a specific pharmacokinetic profile for the different constituents. In fact, at the sampled times, not all the compounds exhibited plasmatic levels coherent with the administered dose. This behaviour can be explained likely due to the different number of constituents and their relative amount observed in the two considered EOs. For example, blood levels of myrcene deriving from the two EOs are comparable at 30 min, even though the administered amount of this compound is four times higher in the MRO compared to the SRO. Limited information is available related to pharmacokinetic studies on myrcene in mice, as most studies have focused on rabbits and rats [24]. In these animals, including rats, an extensive metabolism is reported, such as the biotransformation involving the epoxidation of the 1,2- and 3,10-double bonds, followed by hydration to yield 7-methyl-3-methylene-oct-6-ene-1,2-diol and then 10-hydroxylinalool. These diols were further oxidized, producing their respective aldehydes and hydroxy acids [2].

Table 7
Cecum content of volatile constituents after 90 min of oral administration of MRO and SRO.

	Cecum content MRO 90 min		Cecum content SRO 90 min	
Compound	ng/g	Dev Std	ng/g	Dev Std
α-pinene	218,31	55,00	214,10	53,00
β-pinene	105,66	65,00	99,36	95,00
myrcene	161,59	82,00	36,50	25,00
limonene	145,87	75,00	119,46	85,00
(*Z*)-β-ocimene	18,72	5,00	4,85	15,00
terpinolene	104,03	45,00	57,88	32,00
(*E*)-caryophyllene	1.669,17	169,05	1.744,70	250,00
α-humulene	651,04	62,33	707,64	230,04
cannabidiol			892,00	170,33

Regarding other monoterpenoids, very limited information has been published related to their oral administration, particularly in mice. Some studies have focused on inhalation, and in a previous paper, detectable levels of α- and β-pinene were found in the brain. Pinene levels were 2-fold higher when delivered as an EO, suggesting that uptake of α-pinene by the brain can be influenced by the inclusion of other terpenes [27]. Our data are in agreement with these findings although we used a different route of administration, and our results suggest that a different absorption of the various compounds can occur when complex mixtures of volatiles are orally administered.

Kohlert et al. indicated that for many volatile compounds present in EOs, the elimination route *via* the kidney is prevalent in mice [14]. Our data showed that the volatile compounds exhibit significant tropism for the kidney when administered orally as EO. Therefore, further studies are needed to fully understand this behaviour.

CBD was the most abundant compound in blood at 30 min after SRO administration, and its levels in the liver were also significant at the same sampling time. On the other hand, at 90 min blood and liver levels were strongly decreased namely 30–40 fold probably due to intense phase 2 metabolism of CBD.

Varga et al. studied the effects of (*E*)-caryophyllene on C57BL/6 J mice (10 mg/kg) and reported good oral bioavailability with comparable serum, hepatic, and brain levels [26]. This result appears to be in agreement with our data, although they administered only (*E*)-caryophyllene and not an EO, despite the significantly different doses used.

Comparing the data obtained from the *A. marmelos* EO, despite the differences in doses and EO composition, we observed at least a trend of behaviour showing that brain and liver levels of sesquiterpenoids appear to be higher compared to blood levels at 30 min [19].

The high levels of compounds detected in caecum content at 90 min indicate that further sampling times may be useful to assess possible plasmatic peaks at longer sampling times. We can in fact postulate that the amount present in caecum can act as a possible reservoir of bioactive compounds but this hypothesis needs further studies and may be useful for the development of long release formulations.

5. Conclusions

This study presents an investigation of the tissue distribution of key constituents within two distinct hemp EOs in an animal model. Comprised of low molecular weight, lipophilic, and volatile chemicals, we compared two hemp EOs that we indicated as MRO, dominated by monoterpene constituents, and SRO, rich in less volatile sesquiterpene hydrocarbons, obtained from fresh and dry material, respectively. MRO, which can be obtained from fresh plant material, can be used to administrate monoterpenoids while SRO, which was obtained from dried plant material, can be a valuable source of sesquiterpenoids and CBD. Our findings demonstrate the potential usefulness of hemp EO oral administration, thanks to the easy absorption and the ability to reach multiple tissues. Although the oral use of EOs has been limited thus far, exploring this avenue, particularly with hemp EO, presents an enticing research prospect. Bioactive constituents such as (*E*)-caryophyllene are

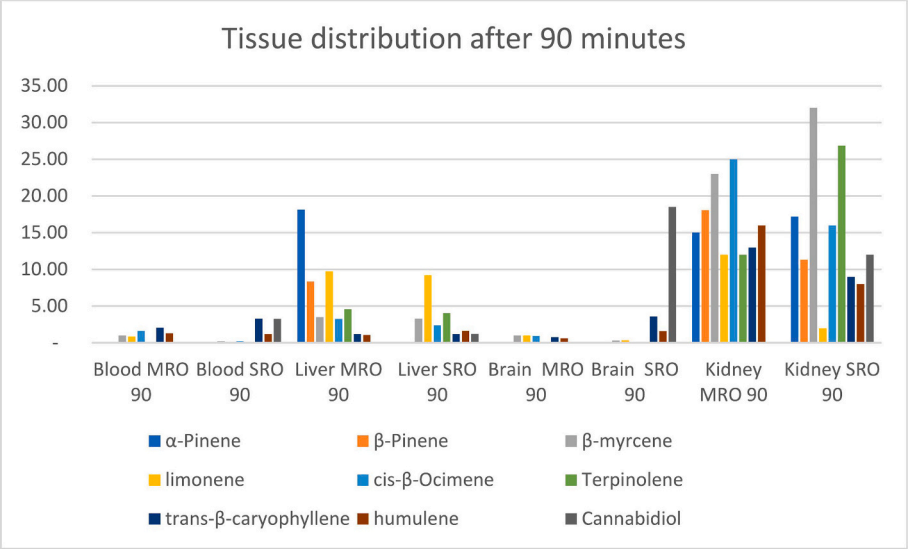

Fig. 2. Levels of analysed volatile compounds from SRO and MRO in blood and tissues of animals after 90 min of oral administration.

being scrutinized for their potential interactions with the CB2 receptor, while the diverse composition of the EO hints at further applications. Our data in mice indicate rapid oral absorption and extensive tissue distribution, including penetration into the brain, suggesting further investigations into the potential use of hemp EO as a constituent of innovative drug formulations. One advantage of EO usage lies in its absence of residual solvents. Moreover, the abundance of both monoterpenes and sesquiterpenes presents new avenues for exploring the pharmacological effects of this product.

This study investigates the tissue distribution of key constituents in two distinct hemp EOs using an animal model. We compared two EOs: MRO, dominated by monoterpenes and obtained from fresh plant material, and SRO, rich in less volatile sesquiterpene hydrocarbons and obtained from dried plant material. Our findings highlight the potential of oral administration of hemp EO due to its efficient absorption and widespread tissue distribution. The results demonstrate that MRO, derived from fresh hemp, is effective for delivering monoterpenoids, while SRO, obtained from dried hemp, serves as a valuable source of sesquiterpenoids and CBD. Notably, the rapid absorption and extensive distribution of these EOs, including brain penetration, underscore their promise as innovative drug formulations. The study also emphasizes the advantage of EOs, which lack residual solvents, and the rich composition of monoterpenes and sesquiterpenes, paving the way for further exploration of their pharmacological effects. In particular, bioactive constituents such as (*E*)-caryophyllene are being investigated for their potential interactions with the CB2 receptor, suggesting new therapeutic applications. These findings open new research avenues for the oral use of hemp EO, positioning it as a promising candidate for further pharmaceutical development. Further studies are warranted to fully explore their clinical potential and mechanisms of action.

CRediT authorship contribution statement

Stefania Sut: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eugenia Mazzara:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Filippo Maggi:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Ignazio Castagliuolo:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Stefano Dall'Acqua:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Riccardo Petrelli:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2024.106147.

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