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Chemical Characterization and Evaluation of the Antimicrobial Activity of Extracts from Two Cultivars of *Cannabis sativa* L. (Tisza and Kompolti) Grown in Sardinia

Claudia Clelia Assunta Juliano [1,*], Ivana Mattu [1], Mauro Marchetti [2] and Marianna Usai [1]

[1] Dipartimento di Medicina, Chirurgia e Farmacia, Università degli Studi di Sassari, Via Muroni 23/a, 07100 Sassari, Italy; i.mattu2@studenti.uniss.it (I.M.); mariannausai54@gmail.com (M.U.)
[2] C.N.R.—Istituto di Chimica Biomolecolare, Traversa La Crucca 3, 07040 Sassari, Italy; mauro@ss.cnr.it
* Correspondence: julianoc@uniss.it

Abstract: The present work was aimed at the chemical characterization and antimicrobial activity of some extracts of aerial parts (essential oils from leaves and inflorescences and resins from inflorescences) of two legal hemp (*Cannabis sativa*) varieties, Tisza and Kompolti, grown in Sardinia. Chemical characterization was carried out by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) techniques. The main constituent was myrcene (11.75% in Tisza and 18.21% in Kompolti); delta-9-tetrahydrocannabinol (THC) was not found, while cannabidiol was present up to 0.36% in Tisza and up to 2.80% in Kompolti. The antimicrobial activity of these extracts against a panel of microorganisms was also determined via minimum inhibitory concentration (M.I.C.) determination. While the results showed minor or negligible antimicrobial activity of the extracts against the Gram+ and *Candida* strains (M.I.C. values equal to or greater than 4 mg/mL), good antibacterial activity (especially of resins) was recorded against *S. aureus* (M.I.C. 0.015–0.031 mg/mL); no substantial differences were detected between the chemical compositions of the two *Cannabis* varieties.

Keywords: *Cannabis sativa* L.; *Cannabis* varieties; Tisza; Kompolti; essential oils; resins; chemical composition; antimicrobial activity; minimum inhibitory concentration (M.I.C.)

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1. Introduction

The objective of the present study was to compare two EU registered hemp cultivars, Kompolti and Tisza, grown in Sardinia, with respect to the composition of their resins and essential oils (EO) and to the antimicrobial activity of these extracts. *Cannabis sativa* is an herbaceous annual plant belonging to the family of *Cannabaceae*, cultivated worldwide since ancient times for medical, recreational, and industrial purposes [1]; it is predominantly dioecious, and this allowed for the hybridization of the plant, leading to thousands of cultivars [2]. The breeding of *C. sativa* strains resulted in over 700 described varieties [3]; on the basis of their content in bioactive compounds, it is possible to distinguish varieties with a high tetrahydrocannabinoids (THC) content from non-THC-producing varieties [3]. Non-THC-producing varieties (THC concentration < 0.2%) are usually referred to as fiber hemp.

C. sativa is a source of fibers, oil, and a wide variety of bioactive compounds synthesized and accumulated in different plant parts; more than 1000 different substances (cannabinoids, terpenes and terpenoids, flavonoids and flavonoid glycosides, polyphenols, and steroids) have been identified [4,5]. Cannabinoids represent the most studied group of metabolites; at present, over 120 phytocannabinoids are known, belonging to 11 classes of a general structure and characterized by a C21 or C22 terpenophenolic skeleton [6]. Some of these compounds are responsible for the psychotropic activity of *Cannabis*, but their pharmacology is quite complex, because many other pharmacological properties are reported [7]. Moreover, extracts of *Cannabis* containing cannabinoids have been reported to exhibit antimicrobial activity, especially against Gram+ bacteria [6,8].

The growing demand from consumers for natural and sustainable beauty products has led to the exploration of the cosmetic potential of *Cannabis*-derived products [2,9,10]. Hemp seed oil is rich in essential fatty acids, with an ideal omega-6/omega-3 rate. It possesses sun protection, skin repair, and anti-aging effects and is considered a high-quality raw material suitable for the production of skincare formulations [11]. Natural cannabidiol (CBD), the most abundant non-psychoactive cannabinoids derived from *C. sativa*, is present both in *Cannabis* extracts and in a low amount in its seed oil (2–20 μg/mL) [12]. In 2021, CBD was included in COSING (Cosmetic Ingredients Database for Information on Cosmetic Substances and Ingredients); it can be used in cosmetic formulations because it possesses anti-inflammatory, antioxidant, and antimicrobial properties against Gram+ bacteria, and it reduces irritation and redness, has potential in acne-prone skin, moisturizes skin, repairs the skin barrier, and slows down aging signs [2,10,13,14].

While most of the studies on *C. sativa* have been focused on cannabinoids, a consistently smaller number of investigations have been carried out on its essential oils (EOs) [15–18]. This essential oil, which can be obtained by using various extraction methods (mainly steam- or hydrodistillation, but also solvent extraction, headspace solid-phase microextraction, and microwave-assisted extraction) [18], is a source of molecules active against different targets of pharmaceutical interest, such as bacteria, enzymes, and cancer cell lines, and is promising for applications in the pharmaceutical, cosmetic, and food industries [16].

Based on these results, the cultivation of legal hemp varieties, especially when carried out in fallow farmlands, can prove to be a source of affordable, biologically active substances, potentially exploitable in various fields of application, then becoming an economically attractive resource.

2. Materials and Methods

2.1. Plants Source

In the present study, we used two Hungarian varieties of *C. sativa* Kompolti and Tisza, obtained from EU-certified seeds, ensuring legal and controlled cultivation. They are characterized by a low content of THC (<0.2%), compliant with European regulations for authorized cultivation, and a medium/high content of cannabidiol (CBD) potential therapeutic or medicinal properties. They are also listed in the EUPVP, the official EU catalog of agricultural plant varieties of agricultural plant species that can be marketed in the EU. Seeds were supplied by the S.O.G. company (Società Agricola Sea of Green, Sassari, Italy). For reference and verification, voucher specimens (dried plant samples) were deposited at Herbarium SASSA of the Department of Medicine, Surgery, and Pharmacy, University of Sassari—Italy.

2.1.1. Cultivation

For the cultivation of *Cannabis*, an experimental field, characterized by clay soil with a slightly alkaline pH value (7.4), was selected near Sassari (North Sardinia), in a valley 70 m above sea level. The place is characterized by a Mediterranean climate with dry summers. The *Cannabis* cultivation was performed in a field uncultivated for several years prior to planting. The soil was superficial ploughing (40–50 cm deep), disc harrowing, and final milling carried out before planting to promote the development of the root system. Seed germination (in a protected environment) was carried out using special containers filled with the growth substrate. The seeds were placed at a depth of 2–3 mm and were covered with a thin layer of peat, maintaining the temperature around 18–25 °C, with a humidity rate of 75–85%, until the roots were developed and well anchored to the substrate. The seedlings were kept in a warm, dark place until the shoot appeared and were immediately planted in the field. The seedlings were planted on the last week of June 2020 at a distance of 1.80 m from each other, leaving a little more in between rows (2 m) (Figure 1). High temperatures characterized the entire growth period, particularly during the first days after transplanting. All summer was characterized by a warm and wet climate, due to the

presence of streams nearby. A self-compensating dripline system was used to water the plants. The use of a self-compensating dripline system of watering had the aim of providing each plant with an equal supply of water, regardless of its position in the field; moreover, this system avoids the waste of water and prevents excessive or insufficient irrigation, conditions that are both unfavorable to plant growth. The frequency of watering and the volume of water supplied to each plant varied at the different stages of plant development, changing from 500 mL every day in the post-implant period to 500 mL on alternate days in the first vegetative phase, and then increasing, with the same frequency, to 1000 mL in the vegetative phase and pre-flowering period and to 2000 mL per day in the advanced stage of flowering. This cultivation method is focused on optimizing root development and providing adequate water management throughout the lifecycle of the plants.

Figure 1. *Cannabis sativa* Kompolti plants 70 days after transplanting.

2.1.2. Monitoring and Harvesting

Plant development was tracked weekly until flowering began. Harvesting occurred when trichomes turned milky white, because the maximum concentration of cannabinoids and terpenes is reached when trichomes take on a milky appearance and their color begins to change to amber. The Tisza variety matured earlier (first decade of October), while the Kompolti variety took longer (last week of October).

Collection method: Aerial parts (flowers and leaves) were collected manually using pruning shears. The material was gathered from the top, sides, and base of the plant for representativeness. Around 3 kg of biomass per variety was collected from various points across the field.

Post-harvest handling: The material was promptly transferred to the lab in a cool container to prevent damage, paying attention so as not to crush it to avoid the loss of volatile compounds. If not immediately processed, the plant material was kept in a freezer at −20 °C.

2.2. Extraction Methods

2.2.1. Extraction of the Essential Oils

The extraction of essential oils was carried out on the leaves and aerial parts of the two varieties of *Cannabis* under consideration. The aerial parts were fully flowered in the flowering stage. Before the extraction of essential oils, earth or other foreign bodies were eliminated, and the plant material was carefully cleaned of any foreign herbs and any parts presenting significant damage or rot. The extraction of essential oils was carried out using a 2 L flask as a boiler, into which the biomass was introduced, and a Clevenger-type apparatus, all in glass (Figure 2). To improve the refrigeration of the steam produced, the refrigerant was connected to a thermostatic bath whose temperature was maintained

around 2 °C to reduce the loss of volatile substances. For each extraction, approximately 800 g of coarsely chopped and uniformly sized biomass was used. The biomass was extracted for approximately 4 h. For each type of sample, three extractions were performed. The collected essential oils were dried on sodium sulfate, transferred to sealed brown glass vials, and stored in the dark at −20 °C until the time of analysis. Extraction yields were calculated as the % of fresh material and are reported in Table 1.

Figure 2. Clevenger apparatus used for the extraction of *Cannabis* essential oils.

Table 1. Yields of essential oil and resin in three replicates' extraction from the hemp varieties Tisza and Kompolti (% of fresh material).

Yield of EO from Leaves Kompolti (% *w*/*w*)	Yield of EO from Leaves Tisza (% *w*/*w*)	Yield of EO from Inflorescences Kompolti (% *w*/*w*)	Yield of EO from Inflorescences Tisza (% *w*/*w*)	Yield of Resin from Inflorescences Kompolti (% *w*/*w*)	Yield of Resin from Inflorescences Tisza (% *w*/*w*)
0.03570	0.01180	0.44700	0.60100	17.70000	19.10000
0.03543	0.01190	0.45120	0.61000	17.54000	19.20000
0.03756	0.01200	0.43870	0.60400	17.32000	18.90000

2.2.2. Extraction of Resins

The resins from the fresh inflorescence material (of the two varieties studied) were obtained by cold extraction on ground material using a stainless-steel extraction apparatus (Roller extractor BHO M150) and liquefied dimethyl ether (DME; Hazchem Chemicals LLC, FZ LUI10 AD01, Jafza South-Mina Jebel Ali-Jabal Ali Industrial Second-Dubai, United Arab Emirates) as a solvent. This technique is used to extract dried and fresh matrices containing oils and resins.

In our experiments, 30–40 g of inflorescences were ground with a grinder. The ground product has been inserted into the extractor, which was carefully closed; on the extractor, there is a valve for inserting the gas cylinder (Figure 3).

The extraction continued until only gas came out. A mixture of the liquefied gas and the phytocomplex was thus obtained.

To remove the gas (DME boiling temperature −24.8 °C), we simply placed the product collection container inside a larger container containing warm water (45 °C) under a chemical hood.

The extracted biomass finally appears as perfectly dried powder. The yields of extraction of the Kompolti variety (17.7%) and Tisza variety (19.1%) were calculated on fresh material and are reported in Table 1.

Safety Concerns: DME is highly flammable and volatile; its use requires extensive safety precautions and specialized equipment. It is vital to understand and follow proper handling and ventilation protocols to avoid accidents and potential health risks, the extraction process involves freezing temperatures; for this reason, it is necessary to use appropriate gloves and protective clothing to prevent injury.

The statistical analysis carried out on the yield of essential oil and resin is reported in Table 2.

Figure 3. Extraction of *Cannabis* resins with the Roller-extractor BHO M150 and dimethyl ether.

2.3. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

2.3.1. GC

To be sure of the reproducibility and to perform statistical analysis, three replicates of each sample of essential oil and resins were analyzed after dilution in n-hexane (solvent non-polar, volatile, suitable for GC) by using a Hewlett–Packard Model 5890A GC, equipped with a flame ionization detector and fitted with a 60 m × 0.25 mm (I.D.), 0.25 µm thickness AT-5 fused silica capillary column (Phenomenex, Torrance, CA, USA). The injection port and detector temperatures were maintained at 280 °C. The column temperature was programmed from 50 °C to 135 °C at 5 °C/min (1 min), 5 °C/min up to 225 °C (5 min), and 5 °C/min up to 260 °C and then held for 10 min. Using this column and the reported temperature program permitted us to perform the better separation of different components based on boiling points. All samples of 0.2 µL (volume injection) were analyzed using 2,6-dimethylphenol and n-tetradecane as internal standards to obtain the correct calibration and accurate quantification. Injection was undertaken using a split/splitless HP 7673 automatic injector and helium as the carrier gas. The injection volume was 0.5 µL, with a split ratio of 1:50. Several measurements of the peak area were performed with an HP

workstation with a threshold set to 0 and a peak width of 0.02. The quantitation of each compound was expressed as the absolute weight percentage using the internal standard and response factors (RFs). The detector RFs were determined for key components relative to 2,6-dimethylphenol and assigned to other components based on the functional group and/or structural similarity, since oxygenated compounds have a lower detectability by an FID (Flame Ionization Detector) than hydrocarbons. The standards (Sigma-Aldrich, Milan, Italy) were >95%, and their actual purity was checked by GC. Several response factor solutions were prepared, which consisted of only four or five components (plus 2,6-dimethylphenol) to prevent interference from trace impurities. We calculated the response factor using a standard mixture of alpha-pinene, alpha-terpineol, nerol, geranial, geranyl acetate, and caryophyllene. The mixture accounted terpenes for 92%, aldehydes for 5%, and alcohols, esters, and sesquiterpenes for 1% each. In our analyses, we obtained a hydrocarbon RF equal to 1, while for alcohols, it was 0.80, and for esters, it was 0.71. For this reason, we multiplied experimental data with the following correction factors: 1 for hydrocarbons, 1.24 for aldehydes and ketones, 1.28 for alcohols, and 1.408 for esters. The use of the above procedure guarantees us high sensitivity and a low probability of involvement with potential interference.

Table 2. Tukey HSD results.

Treatments	Tukey HSD	Tukey HSD	Tukey HSD
Pair	**Q Statistic**	***p*-Value**	**Inference**
A vs. B	0.4221	0.8999947	unsignificant
A vs. C	7.1032	0.0031241	$p < 0.01$
A vs. D	9.8682	0.0010053	$p < 0.01$
A vs. E	303.3435	0.0010053	$p < 0.01$
A vs. F	330.1782	0.0010053	$p < 0.01$
B vs. C	7.5253	0.001942	$p < 0.01$
B vs. D	10.2903	0.0010053	$p < 0.01$
B vs. E	303.7656	0.0010053	$p < 0.01$
B vs. F	330.6003	0.0010053	$p < 0.01$
C vs. D	2.765	0.4196359	unsignificant
C vs. E	296.2404	0.0010053	$p < 0.01$
C vs. F	323.075	0.0010053	$p < 0.01$
D vs. E	293.4754	0.0010053	$p < 0.01$
D vs. F	320.31	0.0010053	$p < 0.01$
E vs. F	26.8347	0.0010053	$p < 0.01$

A = Yield of EO from leaves Kompolti (% w/w); B = Yield of EO from leaves Tisza (% w/w); C = Yield of EO from inflorescences Kompolti (% w/w); D = Yield of EO from inflorescences Tisza (% w/w); E = Yield of Resin from inflorescences Kompolti (% w/w); F = Yield of Resin from inflorescences Tisza (% w/w).

2.3.2. GC/MS

MS analyses were carried out in electron ionization (EI) to permit a correct identification method through the interpretation of the fragmentation pattern. The analysis was carried out with an Agilent Technologies model 7820A connected to a quadrupole MS detector 5977E MSD (Agilent, Santa Clara, CA, USA), using the same conditions and column described above. The column was connected to a mass spectrometer ion source. The injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The ion source temperature was 200 °C. Mass units were monitored from 10 to 900 AMU at 70 eV. For the identification procedure, we considered only peaks from 40 to 900 AMU. The identification of constituents was based on comparisons of Linear Retention index values determined by the comparison of GC retention time to specific compounds (authentic samples) or those reported in the literature [19], and the mass spectra were compared with those obtained from the authentic samples and/or the Nist library spectra or based on the interpretation of the EI fragmentation of the molecules [20] (Table 3).

Table 3. Chemical composition of essential oils and resins from the hemp varieties Tisza and Kompolti.

				EO Flowers TISZA	EO Flowers KOMPOLTI	EO Leaves TISZA	EO Leaves KOMPOLTI	Resin TISZA	Resin KOMPOLTI	[a] IM
Rt	**LRI**	**LRI**	**Compounds**	**% ± SD**	**% ± SD**	**% ± SD**	**% ± SD**	**% ± SD**	**% ± SD**	
12.51	877	879	2-Methyl-1-butyl acetate			0.47 ± 0.02	0.58 ± 0.04			MS-RT
13.05	932	932	α-pinene	13.73 ± 0.11	17.4 ± 0.12	7.28 ± 0.05	11.08 ± 0.11	1.01 ± 0.03	0.81 ± 0.03	Std
13.61	954	955	camphene	0.29 ± 0.02	0.41 ± 0.02					Std
14.63	974	973	β-pinene	5.48 ± 0.04	6.53 ± 0.11	2.74 ± 0.04	4.12 ± 0.07	0.36 ± 0.02	0.27 ± 0.02	Std
15.07	988	992	β-myrcene	56.56 ± 0.14	55.1 ± 0.10	11.75 ± 0.09	18.21 ± 0.21	1.98 ± 0.04	1.45 ± 0.05	Std
16.43	1024	1024	(+)-limonene	6.53 ± 0.07	10.31 ± 0.05	6.92 ± 0.04	7.85 ± 0.06	0.35 ± 0.02	0.33 ± 0.02	Std
16.57	1031	1029	1,8-cineole			0.51 ± 0.10	1.55 ± 0.05			Std
18.75	1050	1051	*cis*-β-ocimene	1.13 ± 0.05	1.04 ± 0.04	1.03 ± 0.04				Std
18.78	1097	1097	linalool			0.14 ± 0.01	1.53 ± 0.05			Std
19.33	1122	1124	exo-fenchol	0,35 ± 0,02	0.52 ± 0.02	0.49 ± 0.02				MS-RT
19.62	1132	1131	allo ocimene	0.3 ± 0.02	0.42 ± 0.02	0.39 ± 0.02				MS-RT
21.75	1189	1187	α-terpineol	0.48 ± 0.03	0.68 ± 0.03	0.65 ± 0.03	0.61 ± 0.03			Std
28.43	1419	1419	*E*-β-caryophyllene	2.83 ± 0.06	1.19 ± 0.04	4.98 ± 0.11	5.37 ± 0.08	0.89 ± 0.06	0.37 ± 0.02	Std
28.69	1443	1444	*Z*-β-farnesene			1.11 ± 0.06	1.12 ± 0.06			Std
29.03	1452	1450	*E*-β-farnesene			0.65 ± 0.04	1.05 ± 0.04			Std
29.32	1455	1455	humulene	0.82 ± 0.04	0.39 ± 0.02	1.57 ± 0.06	1.7 ± 0.05	0.29 ± 0.01	0.23 ± 0.02	Std
29.51	1460	1460	alloaromadendrene			0.97 ± 0.04	0.72 ± 0.04			MS-RT
30.15	1490	1493	β-selinene			1.01 ± 0.04	0.55 ± 0.04			MS-RT
30.36	1498	1499	α-selinene			1.15 ± 0.04	0.61 ± 0.04			MS-RT
30.47	1504	1502	*cis*-β-bisabolene	0.39 ± 0.02		1.38 ± 0.03	2.13 ± 0.06			MS-RT
31.28	1505	1505	*a*-farnesene	0.57 ± 0.04	0.38 ± 0.02	0.78 ± 0.02		0.17 ± 0.01		MS-RT
31.28	1508	1509	α-bisabolene			3.23 ± 0.02	3.41 ± 0.02			MS-RT
31.35	1508	1508	7-epi-α-selinene					0.56 ± 0.04	0.46 ± 0.04	MS-RT
31.36	1508	1509	*trans*-β-guaiene	0.87 ± 0.06	1.22 ± 0.06					MS-RT
31.40	1509	1511	epizonarene			2.61 ± 0.03	1.67 ± 0.04			MS-RT
31.52	1547	1546	selina-3,7(11)-diene	0.95 ± 0.04	1.38 ± 0.05	2.8 ± 0.03	1.66 ± 0.04	0.64 ± 0.04	0.54 ± 0.04	MS-RT
31.89	1583	1583	caryophyllene oxide			4.1 ± 0.05	4.25 ± 0.05			Std
31.91	1587	1585	thujosan-2-α-ol			3.92 ± 0.04	4.04 ± 0.04			MS-RT
31.93	1601	1604	cedrol	0.74 ± 0.06	0.53 ± 0.04					Std
32.77	1601	1604	di-epi-1,10-cubenol					0.69 ± 0.04	0.21 ± 0.02	MS-RT
32.78	1601	1606	guaiol	1.65 ± 0.11	0.5 ± 0.02					Std
33.44	1608	1608	5-epi-7-epi-α-eudesmol	1.64 ± 0.09	0.52 ± 0.04			0.74 ± 0.05	0.23 ± 0.02	MS-RT
33.62	1632	1633	γ-eudesmol	0.34 ± 0.04	0.5 ± 0.03					MS-RT
33.72	1637	1635	cadin-4-en7-ol			5.57 ± 0.05	4.44 ± 0.06			MS-RT
33.82	1640	1640	α-epi cadinol			3.21 ± 0.07	2.59 ± 0.03			MS-RT
33.89	1642	1642	α-muurolol			0.66 ± 0.05				MS-RT
34.12	1651	1650	β-eudesmol	0.69 ± 0.04				0.38 ± 0.02		Std
34.16	1652	1652	cedr-8(15)-en-9-α-ol			8.65 ± 0.12	5.84 ± 0.08			MS-RT
34.17	1654	1653	α-eudesmol	0.81 ± 0.06				0.57 ± 0.02		MS-RT
34.33	1660	1660	selin-11-en-4-α-ol			1.92 ± 0.05	1.23 ± 0.04			MS-RT
34.43	1663	1663	7-epi-α-eudesmol	1.21 ± 0.02	0.44 ± 0.02			0.74 ± 0.05	0.21 ± 0.02	MS-RT
34.66	1674	1677	5-iso-cedranol			9.43 ± 0.10	8.76 ± 0.09	0.74 ± 0.06	0.21 ± 0.02	MS-RT
34.69	1691	1690	*Z*-α-*trans*-bergamotol	1.23 ± 0.03	0.34 ± 0.02					MS-RT
48.81	2375	2375	cannabidiol	0.36 ± 0.02	0.17 ± 0.01	2.8 ± 0.02	0.5 ± 0.04	84.21 ± 0.26	86.48 ± 0.31	MS
49.74	2466	2466	trans-δ-9-tetrahydrocannabinol					0.89 ± 0.04	0.56 ± 0.04	MS
50.37	2520	2520	δ-8-tetrahydrocannabinol					1.68 ± 0.03	2.45 ± 0.03	MS
51.15	2546	2546	cannabigerol					0.75 ± 0.02	1.16 ± 0.02	MS
				99.95	99.97	94.87	97.17	97.74	95.97	

Data are the mean of three replicates ± SD. [a] Identification methods (IM): MS: by comparison of the mass spectrum with those of the computer mass libraries Adams and Nist2011 [19,20] and by interpretation of the mass spectra fragmentations. RI: by comparison of the retention index with those reported in the literature. Std: by comparison of the retention time and mass spectrum of available authentic standards.

2.3.3. Statistical Analysis

The oil yield data were processed via ANOVA to assess whether there are statistically significant differences between the means of multiple groups using MSTAT-C 7.0.1, which is a statistical software package used for various analyses, including ANOVA, and mean separation was performed by the application of Tukey's test (which is a multiple comparison test used to identify which specific groups are significantly different from each other after a significant ANOVA result), with a $p < 0.01$ level of significance. This analysis was conducted to see if there were significant differences in the oil yield between different groups (likely, the two varieties of the plant mentioned earlier). ANOVA was used to test for overall differences, and Tukey's test was used to determine specific groups that differed significantly from each other. The type I error rate is the probability of mistakenly concluding a difference exists when there is none. The chosen significance level of 0.01 indicates that only results with a less than 1% chance of being due to chance will be considered statistically significant.

2.4. Antimicrobial Activity

Stock solutions of the essential oils and of the resins were prepared by dissolving them in DMSO to obtain a concentration of 100 mg/mL (10% *w*/*v*); the solutions were

then sterilized by filtration using sterile membrane filters (Sartorius, pore size 0.22 µm) and stored at −20 °C until use. Preliminary tests with DMSO were performed to ensure that no microorganisms inhibition occurred at the used concentrations.

The antibacterial activity of the *Cannabis* extracts was determined as the Minimum Inhibitory Concentration (M.I.C.) by using a broth microdilution test performed in 96-well microplates, modified with resazurin [21]. This method is based on the use of resazurin dye as a redox indicator: viable bacteria reduce non-fluorescent blue resazurin to the pink fluorescent resorufin; resazurin improves the classical microdilution test, overcoming the problems associated with sparingly soluble products. Resazurin sodium salt (Sigma, Milan, Italy) was dissolved in water at 0.015% *w*/*v*, filter-sterilized (0.22 µm filter), and conserved at 4 °C for no longer than 2 weeks. Microorganisms included both Gram+ (*Staphylococcus aureus* ATCC 6538) and Gram-strains (*Escherichia coli* ATCC 8739) and *Pseudomonas aeruginosa* ATCC 9027). Twofold serial dilutions of mother solutions (ranging from 4 mg/mL to 0.125 mg/mL) were prepared in triplicate in Mueller Hinton Broth (MHB; Oxoid-Thermofisher Scientific, Rodano, Italy) in wells of microplates; control wells contained only the liquid medium. The microplates were inoculated with about 1×10^4 bacteria/well and aerobically incubated at 35 °C for 24 h. After the incubation of microplates for 24 h at 35 °C, 30 µL of resazurin solution was added to each well, and the microplates were further incubated at 35 °C for 2 h. After this time, the plates were visually inspected, and M.I.C. was defined as the lowest concentration of the product at which no color change occurred (Figure 4). To determine the M.B.C. (Minimum Bactericidal Concentration), aliquots of 2 µL of the medium from wells not showing growth were seeded onto Mueller Hinton Agar (MHA; Oxoid-Thermofisher Scientific, Rodano, Italy) plates. After overnight incubation at 35 °C, M.B.C. was defined as the lowest concentration at which no growth was detectable. The results are reported in Table 4.

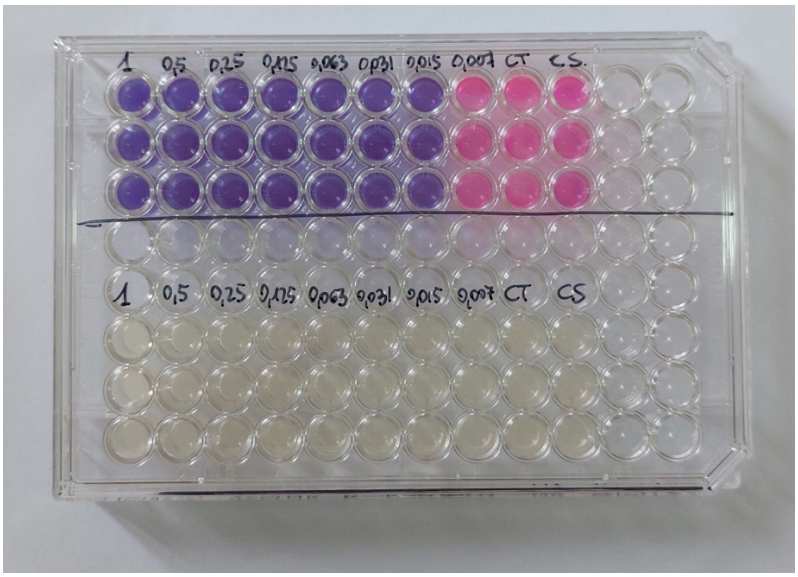

Figure 4. Determination of the M.I.C. of Kompolti resin against *S. aureus* ATCC 6538.

The antifungal activity of the extracts was assessed on *C. albicans* ATCC 10231 by using a plate microdilution test similar to the one described above for bacteria, omitting resazurin. Twofold dilutions of the extracts, ranging from 4 mg/mL to 0.125 mg/mL, were prepared in Sabouraud Liquid Medium (Oxoid-Thermofisher Scientific, Rodano, Italy). The microplates were inoculated with about 1×10^4 yeasts/well and aerobically incubated at 35 °C for 24 h. After incubation, the plates were visually checked for yeast growth, and the M.I.C. was defined as the lowest concentration at which no growth was observed. To determine the M.C.C. (Minimum Candidacidal Concentration), aliquots of 2 µL of medium from each well with no visible growth were subcultured onto Sabouraud Dextrose Agar

(Oxoid-Thermofisher Scientific, Rodano, Italy) plates, which were then incubated at 35 °C for 24 h; the M.C.C. was defined as the lowest concentration at which no growth was detectable. The results are reported in Table 3.

All antimicrobial assays were performed at least in triplicate.

Table 4. Evaluation of the antimicrobial activity of EOs and resins of Tisza and Kompolti hemp varieties. The values (±SD) represent the minimum inhibitory concentrations (MIC) (in brackets, the minimum bactericidal/fungicidal concentrations, MBC/MFC, are shown).

	TISZA	KOMPOLTI
EO from inflorescences	*E. coli* > 4 mg/mL *S. aureus* > 4 mg/mL *Ps. aeruginosa* > 4 mg/mL *Candida albicans* 4 mg/mL (>4 mg/mL)	*E. coli* > 4 mg/mL *S. aureus* > 4 mg/mL *Ps. aeruginosa* > 4 mg/mL *Candida albicans* > 4 mg/mL
EO from leaves	*E. coli* > 4 mg/mL *S. aureus* 0.5 ± 0.0 mg/mL (0.5 ± 0.0 mg/mL) *Ps. aeruginosa* > 4 mg/mL *Candida albicans* 4 ± 0.0 mg/mL (>4 mg/mL)	*E. coli* > 4 mg/mL *S. aureus* 1 ± 0.0 mg/mL (1 ± 0.0 mg/mL) *Ps. aeruginosa* > 4 mg/mL *Candida albicans* 4 ± 0.0 mg/mL (>4 mg/mL)
Resin from inflorescences	*E. coli* > 4 mg/mL *S. aureus* 31 ± 0.0 µg/mL (31 ± 0.0 µg/mL) *Ps. aeruginosa* > 4 mg/mL *Candida albicans* 4 ± 0.0 mg/mL (>4 mg/mL)	*E. coli* > 4 mg/mL *S. aureus* 15 ± 0.0 µg/mL (15 µg/mL) *Ps. aeruginosa* > 4 mg/mL *Candida albicans* 4 ± 0.0 mg/mL (>4 mg/mL)

3. Results and Discussion

3.1. EO and Resins Content (Yield)

The hydrodistillation of frozen inflorescences and frozen leaves gave EO yields of 0.447% and 0.0357% (in the Kompolti variety) and 0.601% and 0.0118% (in the Tisza variety).

The solid–liquid extraction of inflorescences gave a good yield in resin, particularly in the Tisza cultivar (19.1%) (see Table 1).

The statistical analysis carried out on the yield of essential oil and resin in replicates of extraction gave us interesting information, as reported in Table 2. The Honest Significant Differences (HSD) provided by Tukey's test application indicated that there are not significant differences in the yield of essential oil derived from the leaves of both Kompolti and Tisza, and also, there are not significative differences in the yield of essential oil derived from inflorescences coming from Kompolti and Tisza; on the contrary, the differences between the yield in essential oil from the inflorescences and leaves are significant. Also, the difference in the yield of resin between the two cultivars analyzed is really significant, and the Tisza cultivar is richer in resin than the Kompolti cultivar.

3.2. EO Profile of the Two Cultivars

Table 3 shows the chemical composition of the essential oils extracted from the inflorescences (the aerial part, which also had some small leaves in addition to the bracts) and from the leaves of the female plants of the two varieties of *Cannabis*, Tisza and Kompolti, which have distinct chemical profiles. In the oils derived from the flowers, 24 constituents were identified in Tisza and 21 were identified in Kompolti, and there were 10 components with concentrations greater than 1% in Tisza and 8 in Kompolti. Beta-myrcene, alpha-pinene, limonene, and beta-pinene are the most abundant constituents, making up a significant portion of the oils. Beta-myrcene reigns supreme; in fact, this terpene is the main component in both flowers and leaves, although its concentration varies between varieties and plant parts. In flowers, beta-myrcene reaches its highest concentration between 56.56% and 55.10% in the two varieties. Beta-myrcene is a terpene found in the essential oils of

many plants including lemongrass, *Cannabis indica*, and myrcia (*Myrcia sphanocarpa* D.C.; Myrtaceae), from which myrcene takes its name. Myrcene is among the most important chemicals used in perfumes [22]. In order of concentration, we find other interesting terpene components: alpha-pinene (13.73% in Tisza and 17.4% in Kompolti), limonene (6.53% in Tisza and 10.3% in Kompolti), and beta-pinene (5.48% in Tisza and 6.53% in Kompolti). Leaves contain more distinct constituents than flowers, with 32 components identified in Tisza and 27 identified in Kompolti. Also, in these oils, we detected beta-myrcene as the main constituent, but only in the percentage of 11.75% in Tisza and of 18.21% in Kompolti. The other constituents present in high concentrations are: alpha-pinene (7.28% and 11.08% respectively), 5-isocedranol (9.43% and 8.76% respectively), limonene (6.92% and 7.85%), cedr-8(15)-en-9-a-ol (8.65% and 5.84%), and beta-caryophyllene (4.98% and 5.37%). No delta-9-tetrahydrocannabinol (THC), the psychoactive component of *Cannabis*, was found. Cannabidiol was present up to 0.36% in Tisza and up to 2.80% in Kompolti. The different terpene profiles could potentially impact the aroma and therapeutic properties of the essential oils. These compounds have a wide range of biological activities; some of them are interesting in view of the potential use of the essential oils in cosmetic applications, as they exhibit antioxidant (beta-myrcene), anti-inflammatory (beta-myrcene, alpha-pinene, E-beta-caryophyllene), and antibacterial effects (alpha- and beta-pinene, E-beta-caryophyllene, (+)-limonene) [23].

It is worth noting that there are differences from other essential oils of *Cannabis* described in the literature; for instance, the cannabidiol amounts were lower compared to the values reported in the literature for other registered cultivars [18]. On the other hand, these differences should not surprise, because it is widely documented that the composition of *Cannabis* EOs depends on several intrinsic and extrinsic factors, such as the cultivar type, pedoclimatic conditions, harvesting time, processing of plant material before extraction, and extraction techniques [24–26].

3.3. Composition of the Resins

The resin was extracted using dimethyl ether (DME) in a solid liquid extractor. DME is a promising green solvent applicable for the extraction of organic molecules from biomaterials; it has a low boiling point (−23 °C), a medium polarity, and a partial miscibility with water and is a good alternative to conventional solvents because it is safe and environmentally friendly [27]. Using this technique of extraction, we obtained satisfactory yields in resin: 17.7% in the Kompolti cultivar and 19.1% in the Tisza cultivar (Table 1). Table 2 shows the results of the analysis of the resin extracted from the inflorescences of the two *Cannabis* varieties under consideration. From these data, it is possible to observe that we have a high identification rate: over 95% of the constituents in the resin were identified for both varieties. In particular, 97.74% of the constituents present in Tisza and 95.97% of the constituents present in Kompolti were identified. CBD dominates; it is the main component of the resin in both varieties, constituting 84.21% *w*/*w* in Tisza and 86.54% *w*/*w* in Kompolti. There is also a modest quantity of THC; compared to CBD, the THC levels are significantly lower, with delta-9-THC ranging from 0.56% to 0.89% and delta-8-THC ranging from 1.68% to 2.45%. In both varieties, non-psychotropic cannabigerol, normally a minor constituent of *Cannabis*, is present in modest concentrations (0.75% in Tisza and 1.16% in Kompolti). During plant growth, most of the cannabigerol is converted into other cannabinoids, mainly tetrahydrocannabinol (THC) or cannabidiol (CBD), leaving approximately 1% cannabigerol in the plant. There are no other compounds present in high concentrations except for the two terpenes beta-myrcene and alpha-pinene, whose total concentration does not exceed 3% (see Table 3).

3.4. Microbiological Activity

Cannabis EOs (distilled from leaves and inflorescences) have been tested on Gram + and Gram—bacteria and on a *Candida albicans* strain. Overall, the essential oils from inflorescences exhibited no detectable antibacterial activity at the tested concentrations

(MICs are in general >4 mg/mL), while a slight antifungal activity has been shown for *Candida* (MIC of 4 mg/mL) (Table 4). The same mild activity against *Candida* was found for EOs obtained from leaves that, however, also show fair activity against *S. aureus* (MICs 0.5 mg/mL and 1 mg/mL for Tisza and Kompolti EOs, respectively). This activity is bactericidal, as demonstrated by the values of MBCs that are equal to the MICs values. Flower resins have no inhibitory activity against Gram-strains but exhibited interesting activity on *S. aureus* (MIC = MBC 0.015–0.031 mg/mL) (Table 4; Figure 4). It is logical to assume that this activity depends on the high CBD content (84–86%) of resins, since, as already mentioned in the Introduction, the inhibitory activity of CBD against Gram+ pathogens is well documented [6,8,28].

The negligible antimicrobial activity of our EOs should not surprise, because the studies on *Cannabis* EOs demonstrate that their antimicrobial activity is extremely variable. For instance, Iseppi et al. [17] reported the very good activity of six hemp essential oils against Gram-positive bacteria (MIC 1–32 μg/mL), while the same oils proved to be ineffective towards Gram-negative strains. In these EOs the presence of cannabinoids, especially CBD, was also observed, in some samples in quantities up to 1 mg/mL. Since the antimicrobial properties of cannabinoids are well documented [6,8,28], Iseppi et al. concluded that the antimicrobial activity of the analyzed hemp EOs probably arose from a synergism between volatile components and cannabinoids. On the other hand, the EOs of *Cannabis* with different chemical profiles can exhibit modest or poor antibacterial activity [16,29]. Zengin et al. [16] tested the antimicrobial properties of a *Cannabis* EO and reported very high MIC and MBC values (8–16 mg/mL) against different *S. aureus* strains and no activity against yeasts (*Candida* spp., *Malassezia* spp.; MIC > 12,460 μg/mL), while against clinical *Helicobacter pylori*, that EO showed MIC values of 16–64 μg/mL. In general, the high variability of the composition of EO of *C. sativa* documented in the literature may explain the many contradictory data on their antimicrobial activities, even toward the same bacterial species, even if typically, the antibacterial effect is more marked on Gram+ microorganisms compared to Gram− microorganisms, with variable activity on mycetes [30]. In particular, the activity of *C. sativa* extracts against *S. aureus* attracts the attention of researchers because this species not only causes a wide spectrum of infections but can also develop resistance to beta-lactam antibiotics used in hospitals (methicillin-resistant *Staphylococcus aureus* or MRSA) [31,32].

4. Conclusions

Recently, *Cannabis* has gained significant attention in the cosmetic industry due to its beneficial effects on skin health, such as its moisturizing, antioxidant, and anti-inflammatory properties; therefore, it is foreseeable that *Cannabis*-derived skincare products play an increasingly significant role in the cosmetic industry. As a source of valuable cosmetic ingredients, *Cannabis* can generate significant economic value, also considering that this plant requires fewer pesticides, herbicides, and water compared to many conventional crops. In the present study, a characterization of EOs and resins obtained from two hemp varieties (Tisza and Kompolti) was carried out, and their antimicrobial activity toward Gram+ and Gram- bacteria and *Candida* was assessed. Overall, the results obtained in this investigation demonstrate that the resins of the two hemp varieties show interesting activity against *S. aureus*, while the EOs proved to be poorly effective against the microorganisms tested. Considering this selective antimicrobial activity, the non-negligible yields in CBD (raw material increasingly used in medicine and cosmetics), and the multiple environmental benefits of hemp (sustainability, soil phytoremediation, soil structure improvement, drought resistance), the cultivation of these hemp varieties looks promising and profitable, allowing for the exploitation of uncultivated land to produce raw cosmetic materials of good quality.

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