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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

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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 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 a minor or negligible antimicrobial activity of the extracts against Gram+ and *Candida* strains, a good antibacterial activity (especially of resins) was recorded against *S. aureus*; no substantial differences were detected between of the chemical compositions of the two *Cannabis* varieties.

Keywords: *Cannabis sativa* L.; Tisza; Kompolti; essential oils; resins; antimicrobial activity

1. Introduction

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 the hybridization of the plant, leading to thousands of cultivars [2]. 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 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 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 for them: their utility has been investigated in a variety of disorders and diseases, such as chronic pain, nausea and vomiting, spasticity due to paraplegia, epilepsy, depression, anxiety disorder, glaucoma, and inflammatory conditions [7]. Moreover,

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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 explore the cosmetic potential of *Cannabis*-derived products; on the basis of experimental evidence, *C. sativa* is considered at present as a source of attracting cosmetic ingredients, such as seed oil and extracts [2,9,10]. Hemp seed oil is rich in essential fatty acids, with an ideal rate omega-6/omega-3. It possesses sun protection, skin repair and anti-aging effects, and is considered a high-quality raw material suitable for 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 also in its seed oil (2-20 μg/ml) [12]. CBD was included in 2021 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 skin barrier, and slows down aging signs [2,10,13,14].

While most of the studies on *Cannabis 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 application in the pharmaceutical, cosmetic and food industry [16].

Basing 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, and then become an economically attractive resource. Therefore, 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 EOs, and to the antimicrobial activity of these extracts.

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 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 slightly alkaline pH (7.4), was selected near of Sassari (North Sardinia), in a valley 70 m above sea level. The place is characterized by 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 done 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 they have developed and well anchored to the substrate. The seedlings have been kept in a warm, dark place until the shoot appeared and were immediately planted in the

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field. 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). A self-compensating dripline system was used to water the plants. 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.

2.1.2. Monitoring and Harvesting

Plant development was tracked weekly until flowering began. Harvesting occurred when trichomes turned milky white, indicating peak potency. Tisza variety matured earlier (first decade of October), while Kompolti variety took longer (last week of October).

Collection method: Aerial parts (flowers and leaves) were collected manually using pruning shears. Material was gathered from 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: Material was promptly transferred to the lab in a cool container to prevent damage, paying attention not to crush it to avoid the loss of volatile compounds. If not immediately processed 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 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. This procedure is normally followed in all plant material intended for extraction of active ingredients, in order to minimize the possibility of extracting active ingredients that do not correspond to the real composition of the chemical constituents of the plant. In fact, it is known that all plants, but in particular essential ones, react to possible damage or attacks by microorganisms by producing defense substances. The extraction of essential oils was carried out using a 2 L flask as a boiler, into which to introduce the biomass, 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 were used. The biomass was extracted for approximately 4 hours. For each type of sample, three extractions were performed. The collected essential oils were dried on sodium sulphate, transferred to sealed brown glass vials, and stored in the dark at -20°C until the time of analysis. Extraction yields were calculated as % of fresh material and are reported in Table 1.

2.2.2. Extraction of Resins

The resins from 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) using liquefied dimethyl ether, (DME; Hazchem Chemicals LLC–Emirati 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 grams 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 to insert the gas cylinder (Figure 3).

During extraction we worked in a ventilated environment (chemical hood) using a mask to avoid inhaling the gas, safety glasses and wearing an insulating glove to protect the hand because the

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extractor freezes when the gas passes through. The extraction continued until only gas comes out. A mixture of the liquefied gas and the phytocomplex is 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 Kompolti variety (17,7%) and Tisza variety (19.1%) were calculated on fresh material and 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 appropriate gloves and protective clothing to prevent injury.

Table 1. Yields of essential oil and resin from hemp varieties Tisza and Kompolti (% of fresh material).

	KOMPOLTI	TISZA
EO from leaves	**0,0357%**	**0,0118%**
EO from inflorescences	**0,447%**	**0,601%**
RESIN from inflorescences	**17,7%**	**19,1%**

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

2.3.1. GC

To be sure of 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 x 0.25 mm (I.D.), thickness 0.25 μm 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), 5°C/min up to 260°C, and then held for 10 min. Using this column and the reported temperature program permit 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 get the correct calibration and accurate quantification. Injection was undertaken using a split/splitless HP 7673 automatic injector and helium as carrier gas. Several measurements of peak area were performed with a HP workstation with a threshold set to 0 and peak width 0.02. Quantization of each compound was expressed as absolute weight percentage using the internal 187 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 functional group and/or structural similarity, since oxygenated compounds have a lower detectability by FID (Flame Ionization Detector) than hydrocarbons. Standards (Sigma-Aldrich, Fluka and Merck grade) were >95%, and their actual purity was checked by GC. Several response factor solutions were prepared that consisted of only four or five components (plus 2,6- dimethylphenol) to prevent interference from trace impurities. It is known that oxygenated compounds have a lower sensitivity than hydrocarbons to FID. 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 5%, and alcohols, esters, and sesquiterpenes 1% each. In our analyses we obtained a hydrocarbon RF equal to 1, while for alcohols it was 0.80, and for esters 0.71. For this reason, we multiplied experimental data with the following correction factors: 1 for

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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.

2.3.2. GC/MS

MS analyses were carried out in E.I. to permit a correct identification methos through the interpretation of fragmentation pattern. The analysis was carried out with an Agilent Technologies model 7820A connected to a quadrupole MS detector 5977E MSD (Agilent), using the same conditions and column described above. The column was connected to a mass spectrometer ion source. 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. Identification of constituents was based on comparisons of Kovat's index values determined by comparison of GC retention time to specific compounds (authentic samples) or those reported in literature [19] and mass spectra with those obtained from the authentic samples and/or Nist library spectra or based on interpretation of the EI fragmentation of the molecules [20] (Table 2).

2.3.3. Statistical Analysis

Oil yield data were processed via ANOVA to assesses whether there are statistically significant differences between the means of multiple groups using MSTAT-C which is a statistical software package used for various analyses, including ANOVA, and 170 mean separation was performed by application of Tukey's test (that is a multiple comparison test used to identify which specific groups are significantly different from each other after a significant ANOVA result) with p 0.05 level of significance, this means that only differences with a probability of occurring by chance less than 5% are considered. This analysis was conducted to see if there were significant differences in oil yield between different groups (likely the two varieties of 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 chosen significance level of 0.05 implies that only very strong evidence will be considered statistically significant. Tukey's Test, also known as Tukey's Honest Significant Difference (HSD) test, is a post-hoc multiple comparison test used after a significant ANOVA result. It identifies which specific pairs of groups differ significantly from each other, controlling for the overall. Type I error rate (the probability of mistakenly concluding a difference exists when there is none). The chosen significance level of 0.05 indicates that only results with a less than 5% 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/vol.); 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 assure that no microorganisms inhibition occurred at used concentrations.

The antibacterial activity of the *Cannabis* extracts was determined as 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) was dissolved in water at 0.015% w/vol, filter sterilised (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 liquid medium. Microplates were

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inoculated with about 1 x 10^4 bacteria/well and aerobically incubated at 35 C for 24 h. After the incubation of microplates for 24 hours at 35°C, 30 µl of resazurin solution were added to each well, and microplates were further incubated at 35°C for 2 hours. After this time, the plates were visually inspected and M.I.C. was defined as the lowest concentration of product at which no colour change occurred (Figure 4). To determine the M.B.C. (Minimum Bactericidal Concentration), aliquots of 2 µL of 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. Results are reported in Table 3.

The antifungal activity of the extracts was assessed on *C. albicans* ATCC 10231 by using a plate microdilution test similar to that 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). Microplates were inoculated with about 1x10^4 yeasts/well and aerobically incubated at 35 °C for 24 h. After incubation, 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; M.C.C. was defined as the lowest concentration at which no growth was detectable. Results are reported in Table 3.

All antimicrobial assays were performed at least in triplicate.

Table 3. Evaluation of the antimicrobial activity of EOs and resins of Tisza and Kompolti hemp varieties. The values represent the minimum inhibitory concentrations (MIC) (in brackets the minimum bactericidal/fungicidal concentrations, MBC/MFC).

	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 mg/ml (0.5 mg/ml) *Ps.aeruginosa* > 4 mg/ml *Candida albicans* 4 mg/ml (> 4 mg/ml)	*E.coli* > 4 mg/ml *S. aureus* 1 mg/ml (1 mg/ml) *Ps.aeruginosa* > 4 mg/ml *Candida albicans* 4 mg/ml (> 4 mg/ml)
Resin from inflorescences	*E.coli* > 4 mg/ml *S. aureus* 31 µg/ml (31 µg/ml) *Ps.aeruginosa* > 4 mg/ml *Candida albicans* 4 mg/ml (> 4 mg/ml)	*E.coli* > 4 mg/ml *S. aureus* 15 µg/ml (15 µg/ml) *Ps.aeruginosa* > 4 mg/ml *Candida albicans* 4 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 Kompolti variety) and 0,601% and 0,0118% (in Tisza variety).

The solid-liquid extraction of inflorescences gave a good yield in resin, particularly in Tisza cultivar (19,1%) (see Table 1).

3.2. EO Profile of the Two Cultivars

Table 2 shows the chemical composition of the essential oils extracted from the inflorescences (aerial part which also had some small leaves in addition to the bracts) and from the leaves only of the female plants of the two varieties of Cannabis, Tisza and Kompolti, which have distinct chemical

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profiles. In the oils derived from the flowers, 24 constituents were identified in Tisza and 21 in Kompolti, being 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 reach 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. In order of concentration, we find other significant 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 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 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 a potential use of the essential oils in topical a cosmetic application, 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) [22].

It is worth noting that there are differences from other essential oils of *Cannabis* described in the literature; for instance, 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 cultivar typo, pedo-climate conditions, harvesting time, processing of plant material before extraction, and extraction techniques [23–25].

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, a partial miscibility with water, and is a good alternative to conventional solvents because it is safe and environmentally friendly [26]. Using this technique of extraction, we obtained satisfactory yields in resin: 17,7% in the Kompolti cultivar and 19,1% in 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, constitutes 84.21% in Tisza and 86.54% in Kompolti. There is also a modest quantity of THC; compared to CBD, 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 2).

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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 3). The same mild activity against *Candida* was found for EOs obtained from leaves that, however, also show a 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 values that are equal to MICs values. Flower resins have no inhibitory activity against Gram- strains, but exhibited an interesting activity on *S. aureus* (MIC = MBC 0.015-0.031 mg/ml) (Table 3). It is logical to assume that this activity depends on the high CBD content (84-86%) of resins, since, as already mentioned in Introduction, the inhibitory activity of CBD against Gram+ pathogens is well documented [6,8,27].

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 a 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 [6,8,27], Iseppi *et al.* concluded that the antimicrobial activity of analysed hemp EOs probably arose from a synergism between volatile components and cannabinoids. On the other hand, EOs of *Cannabis* with different chemical profiles can exhibit a modest or poor antibacterial activity [16,28]. 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 >12460 μg/ml), while against clinical *Helicobacter pylori* that EO showed MIC values of 16-64 μg/ml.

Conclusions

Recently, *Cannabis* has gained significant attention in the cosmetic industry due to its beneficial effects on skin health, such as moisturizing, antioxidant, anti-inflammatory properties; therefore, it is foreseeable that *Cannabis*-derived skincare products play an increasingly significant role in cosmetic industry. As 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 an interesting activity against *S. aureus*, while the EOs proved to be poorly effective against the microorganisms tested. Considering this selective antimicrobial activity, the not 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) [29], the cultivation of these hemp varieties looks promising and profitable, allowing to exploit uncultivated land to produce raw cosmetic materials of good quality.

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