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Exploring the Diverse Biological Properties of Cannabidiol: A Focus on Plant Growth Stimulation

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The aim of the current study was to compare some biological activities of edible oils enriched with 10% of cannabidiol (CBD samples) from the Slovak market. In addition, hemp, coconut, argan, and pumpkin pure oils were also examined. The study evaluated the fatty acids content, as well as antibacterial, antifungal, antioxidant, cytotoxic, and phytotoxic activities. The CBD samples presented antimicrobial activity against the tested bacterial strains at higher concentrations (10000 and 5000 mg/L) and antifungal activity against *Alternaria alternata*, *Penicillium italicum* and *Aspergillus flavus.* DPPH• and FRAP assays showed greater activity in CBD-supplemented samples compared to pure oils and vitamin E. In cell lines (IPEC-J2 and Caco-2), a reduced cell proliferation and viability were observed after 24 hours of incubation with CBD samples. The oils showed pro-germinative effects. The tested activities were linked to the presence of CBD in the oils.

Introduction

Natural plant products have been used for centuries for various purposes in medicine, the food industry, perfumery, or agro-industry. *Cannabis sativa* L. (Cannabaceae) was one of the earliest plants cultivated by humans for fibers as far back as 4,000 BC. The medicinal use of cannabis was reported in the world oldest Pharmacopoeia.[1] In India, the Atharva Veda (a collection of sacred texts by an unknown author) mentions cannabis as one of the five sacred plants, describing it as a source of happiness, a giver of joy, and a bringer of freedom.[2] Consequently, the use of cannabis became a part of numerous religious rituals in that region.[2] Subsequently, its psychoactive properties were recognized, leading to its use as a recreational, medicinal, and spiritual drug.[3]

In the past, *Cannabis* was primarily cultivated for its fiber, but in recent times, the plant has garnered significance in the medicinal field owing to its production of unique cannabinoids.[4] The latter half of the 20th century witnessed a surge in the societal impact of cannabis due to a notable increase in its consumption. Since 1965, there has been a marked rise in scientific exploration of cannabis, particularly focusing on its main constituents. The number of publications on cannabis peaked in the early 1970s. Noteworthy contributions during this era were made by a Brazilian research team under Carlini's leadership, particularly in elucidating the interactions of Δ9-THC (Δ9-tetrahydrocannabinol) with other cannabinoids.[5] The interest in cannabis studies was renewed in the early 1990s with the identification and cloning of specific cannabinoid receptors in the Nervous System, along with the discovery of anandamide, an endogenous cannabinoid.[6] The two predominant cannabinoids in the hemp plant are tetrahydrocannabinol (THC) and cannabidiol (CBD). On average, the hemp plant contains 12–18% CBD and 0.3% THC.[7] Cannabis contains varying concentrations of more than 100 naturally occurring phytochemicals.[8] The hemp seeds are rich in nutrients, containing 25–35% lipids, 20–25% proteins, and 30% carbohydrates. They also contain 50–70% linoleic acid (LA, an omega-6 fatty acid) and 15–25% α-linolenic acid (ALA, an omega-3 fatty acid), maintaining a beneficial 3:1 ratio, the optimal for human nutritional requirements. Additionally, hemp seeds provide other polyunsaturated fatty acids (PUFAs), like gamma-linolenic acid (GLA).[9] The diverse biological properties of the plant are attributed to its rich array of phytochemicals, including tocopherols (100–150 mg/100 g of oil), terpenes, phenolic compounds, cannabinoids, vitamins, and minerals.[10]

CBD was identified in hemp essential oil (EO) extracted from various parts of the plant such as leaves, flowers, seeds, seed bracts, inflorescences, and thinner stems of the plant.[11] Some researchers[12] suggested that non-psychoactive cannabinoids

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like CBD are mainly present in seed oil. CBD exhibited several biological activities, such as antimicrobial and antioxidant.[13,14]

Edible oils enriched with 5% to 30% CBD have gained significant popularity among the general population in recent years. The market for these products is dynamic, with a multitude of producers and variations available. The utilization of these oils is primarily motivated by their effects on the human and animal nervous systems. The objective of this comprehensive research was twofold: 1) to compare similar products from various producers and assess them through diverse bioassays, and 2) to uncover potential biological activities of the product that could be harnessed for various applications independently. This indicates that cannabidiol, as a natural product, may possess undiscovered biological activities beyond its known impact on the nervous system. The aim of this study was to expand the knowledge on the potential antioxidant, cytotoxic, antimicrobial, and phytotoxic effects of some commercially available edible oils enriched with 10% CBD.

Results and Discussion

Fatty Acid Composition

The presented research focused on testing the biological activity of edible oils enriched with 10% CBD. Along with the mentioned samples, pure edible oils without CBD were also tested. The results than compared the biological activity of the oils with and without CBD. Table 1 presented the fatty acid (FA) composition of the four pure seed oils analysed. The data agreed with literature data, although in some cases the percentages of components were different. Pure argan oil (pAO) contained mainly unsaturated fatty acids (83.8%), with oleic acid being the primary compound at 54.0%, followed by linoleic acid (22.9%). Saturated fatty acids, on the other hand, were primarily represented of palmitic acid (12.7%). This composition agreed with the literature.[15,16] Pure coconut oil (pCO) presented the highest percentage of saturated fatty acids (SFA) (42.7%): caprylic (11.2%), myristic (28.8%) and palmitic acids (2.7%) have also been identified. Unsaturated fatty acids (UFAs) were characterized by oleic (46.5%) and linoleic acids (1.7%). Due to this abundant presence of SFA, the ratio saturated/unsaturated fatty acids were the highest (0.8) among those tested. The literature data were slightly different from those obtained in the present research.[17] In fact, the oil analysed by Marina and collaborators[18] had a high quantity of lauric acid, missing in the sample tested, and a lower quantity of oleic acid compared to the sample under examination (5.2 *vs* 46.5%).

Pure pumpkin oil (pPO) was particularly rich in UFAs (83.1%), the most abundant being oleic acid (41.3%), followed by linoleic (34.6%) and linolenic acids (6.2%). These data agree with the literature.[19] Pure hemp seed oil (pHO) was characterized by several SFAs such as palmitic (7.9%), stearic (7.4%), arachidic (1.2%) and behenic acids (0.9%); UFAs mainly consisted of oleic (53.7%), linoleic (21.7%), linolenic (3.1%) and gadoleic acids (1.7%). The data obtained present some differences with the literature. In fact, the percentage of linoleic acid, in some papers, was higher than that of oleic acid. This difference may be attributed to the utilization of different separation techniques. Da Porto and colleagues noted that polyunsaturated fatty acids (PUFAs), such as linoleic acid, were more effectively extracted using supercritical carbon dioxide fluid extraction.[20] The ratios of unsaturated fatty acids (UFAs) to saturated fatty acids (SFAs) found in pure argan, coconut, pumpkin, and hemp oils were 5.5, 1.1, 6.1, and 4.6 respectively. Additionally, the ratio between omega-6 and omega-3 fatty acids in the samples was assessed. Hemp oil exhibited the highest ratio at 7.0 compared to argan and pumpkin oil at 3.9 and 5.9 respectively; this value could not be calculated for coconut oil. The recommended dietary intake for the omega-6 to omega-3 ratio is around 1:1 or 4:1; currently, the British diet

Table 1. The composition of fatty acid (%) in pure argan, coconut, pumpkin and hemp oils.

	pAO(%)	pCO (%)	pPO (%)	pHO (%)
Caprylic acid (C8:0)	–	11.2±0.3	–	–
Myristic acid (C14:0)	–	28.8±0.6	–	–
Palmitic acid (C16:0)	12.7±0.2	2.7±0.1	12.7±0.5	7.9±0.4
Palmitoleic acid (C16:1)	–	–	0.1±0.1	0.1±0.0
Stearic acid (C18:0)	–	–	–	7.4±0.5
Oleic acid (C18:1)	54.0±0.5	46.5±0.8	41.3±0.8	53.7±0.8
Linoleic acid (C18:2)	22.9±0.3	1.7±0.12	34.6±0.6	21.7±0.5
Linolenic acid (C18:3)	5.9±0.1	-	6.2±0.3	3.1±0.1
Arachidic acid (C20:0)	1.1±0.0	-	0.6±0.0	1.2±0.0
Gadoleic acid (C20:1)	1.0±0.1	-	0.9±0.1	1.7±0.1
Behenic acid (C22:0)	0.4±0.0	-	0.4±0.0	0.9±0.2
Lignoceric acid (C24:0)	–	–	–	0.1±0.1
Total	98.0	93.6	96.8	97.6
Saturated fatty acids	14.2	42.7	13.7	17.5
Unsaturated fatty acids	83.8	50.9	83.1	80.1
Ratio unsaturated/saturated	5.5	1.1	6.1	4.6
Ratio omega-6/omega-3	3.9	–	5.9	7.0

*Notes: pAO = pure argan oil, pCO = pure coconut oil, pPO = pure pumpkin oil, pHO = pure hemp oil. The experiments were performed in triplicate and the results are expressed as mean ± SD.

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provides these fatty acids in a ratio of 7:1, which aligns with the ratio observed in the hemp sample discussed in this study.[21] These values promote their possible use in the field of the human health, in particular in the prevention of events related to the cardiovascular system.[21] In fact, the introduction of these oils into the diet, as they are characterized by a high UFA/SFA ratio and good ω-6/ω-3 ratio, could reduce atherogenic and thrombogenic events.[22,23]

Antibacterial Activity

Edible oils enriched with CBD, as well as pure edible oils, were subjected to testing for their potential antibacterial activity. The assessment included four types of pure oils: coconut oil enriched with CBD from three different companies (e1CO, e2CO, and e3CO), hemp oil enriched with CBD from two companies (e1HO and e2HO), argan oil enriched with CBD from one company (eAO), and pumpkin oil enriched with CBD from another (ePO). The standard disc diffusion method was employed, using model microorganisms such as *Pseudomonas fluorescens* Flügge, *Pseudomonas aeruginosa* Schröter (Migula), *Bacillus cereus* Frankland & Frankland, *Bacillus megaterium* de Bary, *Bacillus mojavensis* Roberts, *Xanthomonas campestris* Pammel, and *Xanthomonas vesicatoria* Doidge. All the studied samples had antibacterial activity against the majority of the tested bacterial strains equal to or higher than the positive controls (Tetracycline and Streptomycin), as illustrated in Table 2. None of the CBD samples showed activity against *P. aeruginosa*. ePO showed the most significant activity against *P. fluorescens*, *B. megaterium* and *X. vesicatoria*, at the highest tested concentration (10000 mg/L); also, the concentration of 5000 mg/L showed effective antibacterial activity. In addition, e2HO and eAO displayed the most significant activity against *P. fluorescens* at the highest tested concentration, with only eAO showing an effect against the same bacterium even at 5000 mg/L. e1HO and e3CO demonstrated the highest significant activity against *X. campestris* and *X. vesicatoria* only at the highest tested concentration. Two samples, e1CO and e2CO, displayed high activity against *X. vesicatoria* only at the highest tested concentration (10000 mg/L). On the other hand, e1HO and e3CO showed no effect against all tested bacteria at 5000 mg/L. None of samples showed any effect against all bacteria tested at the lowest concentration (1000 mg/L). Moreover, the pure oils resulted inactive when tested for the antimicrobial activity.

Cannabidiol is a compound of considerable interest, due to its properties and lack of psychotropic effects.[24] The antimicrobial activity of CBD against Gram-negative (G-ve) bacteria is dependent on lipopolysaccharides (LPS) present in the cellular membrane structure.[11] In fact, various types of LPS can be found in different genera of G-ve bacteria, constituting approximately 80% of their outer cell membrane. A recent study demonstrated that the limited activity of CBD against most G-ve bacteria is due to both the outer membrane and lipopolysaccharide.[25] In fact, the ability of the compound to

Table 2. Antibacterial activity of edible oils enriched with CBD, tetracycline and streptomycin.

Tested bacteria		e1CO	e2CO	e3CO	e1HO	e2HO	eAO	ePO	Tetracycline	Streptomycin
P. fluorescens	C1	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	1.4 ± 0.4^{b}	2.3 ± 0.2^{a}	2.4 ± 0.1^{a}	2.8 ± 0.2^{a}	3.5 ± 1.0^{a}	2.5 ± 0.7^{a}
	C2	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	1.8 ± 0.2^{c}	1.2 ± 0.2^{c}	2.0 ± 0.2^{b}		
	C3	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}		
B. megaterium	C1	0.80 ± 0.1^{c}	1.7 ± 0.1^{ab}	0.0 ± 0.0^{d}	1.2 ± 0.6^{b}	1.4 ± 0.2^{b}	1.4 ± 0.6^{b}	2.7 ± 0.1^{ab}	4.0 ± 1.6^{a}	1.9 ± 0.4^{ab}
	C2	0.30 ± 0.1^{c}	1.2 ± 0.2^{b}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.9 ± 0.2^{b}	0.9 ± 0.1^{c}	1.6 ± 0.1^{b}		
	C3	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}		
B. cereus	C1	1.7 ± 0.2^{c}	2.2 ± 0.2^{b}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	1.3 ± 0.2^{c}	1.8 ± 0.1^{c}	0.6 ± 0.2^{d}	4.5 ± 0.9^{a}	1.5 ± 0.4^{c}
	C2	1.2 ± 0.1^{c}	1.7 ± 0.2^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.8 ± 0.1^{c}	1.3 ± 0.2^{c}	0.0 ± 0.0^{e}		
	C3	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}		
X. campestris	C1	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	2.4 ± 0.2^{ab}	3.0 ± 0.3^{a}	0.0 ± 0.0^{c}	0.6 ± 0.1^{b}	0.0 ± 0.0^{c}	3.8 ± 0.9^{a}	2.7 ± 0.7^{ab}
	C2	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}		
	C3	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}		
X. vesicatoria	C1	2.0 ± 0.6^{b}	2.1 ± 0.1^{b}	2.9 ± 0.3^{b}	1.9 ± 0.2^{bc}	1.4 ± 0.2^{bc}	0.9 ± 0.1^{c}	2.5 ± 0.2^{b}	4.2 ± 0.9^{a}	2.4 ± 0.3^{b}
	C2	0.9 ± 0.2^{c}	1.6 ± 0.1^{bc}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}		
	C3	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}		
C. michiganensis	C1	1.3 ± 0.2^{b}	1.1 ± 0.1^{b}	1.0 ± 0.6^{bc}	0.9 ± 0.1^{bc}	1.2 ± 0.1^{b}	1.8 ± 0.5^{b}	0.9 ± 0.2^{bc}	3.5 ± 0.5^{a}	1.9 ± 0.4^{b}
	C2	0.6 ± 0.1^{c}	0.4 ± 0.1^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.5 ± 0.2^{c}	1.1 ± 0.3^{bc}	0.2 ± 0.0^{c}		
	C3	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}		

Where: C1, C2 and C3 are the tested concentrations 10000, 5000 and 1000 mg/L on samples where e1CO, e2CO and e3CO are coconut oil enriched by CBD from three different companies, e1HO and e2HO are hemp oil enriched by CBD from two companies, eAO is argan oil enriched by CBD from one company and ePO pumpkin oil enriched by CBD from one company. Values followed by different letters for each tested bacterium are. significantly different according to Tukey post host test with probability $P\leq0.05$ using SPSS statistical program. Data are expressed as mean values ± SDs.

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Table 3. Antifungal activity of edible oils enriched with CBD.

Samples	Fungal mycelium inhibition (%)		
	A. alternata		
	C1	C2	C3
e1CO	46.9±0.1^{c}	24.4±0.2^{d}	11.2±0.2^{de}
e2CO	44.8±0.2^{c}	36.7±0.1^{c}	32.6±0.4^{cd}
e3CO	46.9±0.1^{c}	38.7±0.1^{c}	10.2±0.2^{de}
e1HO	61.2±0.1^{b}	22.4±0.4^{d}	3.0±0.3^{e}
e2HO	39.7±0.0^{c}	32.6±0.2^{cd}	3.1±0.1^{e}
eAO	71.4±0.1^{a}	60.2±0.1^{b}	53.1±0.2^{c}
ePO	72.4±0.1^{a}	61.2±0.1^{b}	34.6±0.2^{cd}
C-ve	0.0±0.0^{e}		
C+ve	79.5±0.3^{a}		
	B. cinerea		
	C1	C2	C3
e1CO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
e2CO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
e3CO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
e1HO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
e2HO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
eAO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
ePO	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
C-ve	0.0±0.0^{b}		
C+ve	81.0±0.3^{a}		
	P. italicum		
	C1	C2	C3
e1CO	45.0±0.3^{b}	24.0±0.3^{bc}	3.0±0.2^{e}
e2CO	0.0±0.0^{e}	0.0±0.0^{e}	0.0±0.0^{e}
e3CO	30.0±0.3^{b}	6.9±0.2^{ce}	0.0±0.0^{e}
e1HO	36.0±0.3^{b}	14.0±0.2^{c}	0.0±0.0^{e}
e2HO	41.0±0.1^{b}	17.0±0.2^{c}	0.0±0.0^{e}
eAO	16.0±.0.3^{c}	9.0±0.3^{ce}	0.0±0.0^{e}
ePO	21.0±0.2^{c}	13.0±0.2^{ce}	0.0±0.0^{e}
C-ve	0.0±0.0^{e}		
C+ve	73.0±0.2^{a}		
	A. flavus		
	C1	C2	C3
e1CO	52.0±0.1^{b}	22.0±0.1^{c}	0.0±0.0^{d}
e2CO	26.0±0.1^{bc}	13.0±0.1^{c}	0.0±0.0^{d}
e3CO	34.0±0.2^{bc}	17.0±0.1^{c}	0.0±0.0^{d}
e1HO	73.0±0.2^{ab}	48.0±0.1^{b}	12.0±0.2^{c}
e2HO	22.0±0.1^{c}	0.0±0.0^{d}	0.0±0.0^{d}
eAO	14.0±0.2^{c}	0.0±0.0^{d}	0.0±0.0^{d}
ePO	74.0±0.2^{ab}	50.0±0.3^{b}	14.0±0.2^{c}
C-ve	0.0±0.0^{d}		
C+ve	95.0±0.2^{a}		

Where: C1, C2 and C3 are the tested concentrations 10000, 5000 and 1000 mg/L on samples where e1CO, e2CO and e3CO are coconut oil enriched by CBD from three different companies, e1HO and e2HO are hemp oil enriched by CBD from two companies, eAO is argan oil enriched by CBD from one company and ePO pumpkin oil enriched by CBD from one company. C-ve: negative control; C+ve positive control. Values followed by different letters for each tested fungi are significantly different according to Tukey post host test with probability $P \leq 0.05$ using SPSS statistical program. Data are expressed as mean values±SDs.

Table 4. Antioxidant activity of pure oils and oils enriched with CBD

	DPPH$^{\bullet}$ IC$_{50}$(mg/ml) (Mean±SD)	FRAP µmol Fe^{2+} equivalents/g oil (Mean±SD)
pPO	10.8±0.3^{bc}	35.2±0.9^{e}
pAO	12.5±1.4^{b}	19.2±0.7^{e}
pCO	33.4±4.8^{a}	52.8±0.5^{e}
pHO	7.0±0.4^{c}	38.3±0.2^{e}
e1CO	0.4±0.0^{d}	161.5±1.4^{bc}
e2CO	0.5±0.0^{d}	157.8±1.7^{c}
e3CO	0.3±0.0^{d}	194.4±0.6^{ab}
e1HO	0.4±0.0^{d}	161.4±0.5^{bc}
e2HO	0.5±0.0^{d}	208.2±0.3^{a}
eAO	0.3±0.0^{d}	224.1±1.0.^{a}
ePO	0.34±0.0^{d}	226.0±0.4^{a}
Vitamin E	6.85±0.13^{c}	±3.0^{d}

*Samples: pAO=pure argan oil, pCO=pure coconut oil, pPO=pure pumpkin oil, pHO=pure hemp oil, e1CO, e2CO and e3CO are coconut oil enriched by CBD from three different companies, e1HO and e2HO are hemp oil enriched by CBD from two companies, eAO is argan oil enriched by CBD from one company and ePO pumpkin oil enriched by CBD from one company. Values followed by different letters in each column are significantly different according to Tukey post host test with probability $P \leq 0.05$ using SPSS statistical program. Data are expressed as mean values±SDs.

destroy cell membranes and the low concentration of LPS might make G-ve bacteria more susceptible to the effects of CBD. In the bibliographic research, the antimicrobial activity against *Staphylococcus aureus* may be linked to some single constituents such as CBD.[26]

Antifungal Activity

Potential antifungal activity was evaluated against the phytopathogenic fungi *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers., *Penicillium italicum* Wehmer, and *Aspergillus flavus* Link., using the incorporation method.

All samples tested exhibited antifungal activity against *A. alternata*, *P. italicum* and *A. flavus*, as illustrated in Table 3. No activity against *B. cinerea* was observed. Regarding *A. alternata*, the samples eAO and ePO, showed a significant activity at the concentration of 10000 mg/L, while, e1HO at 10000 mg/L, and both eAO and ePO, at 5000 mg/L, showed moderate activity. On the other hand, e1HO at the lowest concentration, showed the least effect; e2HO, at the same lowest concentration,

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showed no effect. For *P. italicum*, all tested samples, except e2CO, showed a moderate effect compared to the positive control; e1CO, e1HO, e2HO and e3CO showed significant effect, at the highest concentration. Regarding *A. flavus*, e1HO and ePO samples showed dose dependent activity particularly at 10000 mg/L, showing slightly lower but strong activity compared to the positive control. Furthermore, the same samples, also displayed a moderate effect even at 5000 mg/L. Moreover, pure oils tested for antifungal activity, were found to be inactive. Notably, other studied[27] reported bioactivity of hemp oil against some *Candida* species such as *C. tropicalis, C. albicans* and *C. krusei*.

Antioxidant Activity

The antioxidant activity was evaluated using two different tests: DPPH• and FRAP. The DPPH• test measures the substance's reducing capacity, whether through hydrogen transfer or electron transfer. However, the limits of this analytical technique are in the presence of large sterically voluminous molecules, which are not able to react with the reactive part of the radical.

The FRAP test allowed the evaluation of the reducing capacity only by electron transfer.[28] The results showed that CBD-spiked samples demonstrated higher antioxidant activity than the pure oils (Table 4).

The antioxidant activity of CBD is attributed to the presence of two hydroxyl groups.[29] Furthermore, the presence of various resonance structures gives this substance a potential antioxidant activity due to the free radicals are mainly distributed on the ether and alkyl groups, along with the benzene ring.[30]

In the DPPH• assay, the CBD-spiked samples presented a significant increase in antioxidant power (<0.6 mg/ml) than positive control (Vitamin E). The pure oils presented the highest IC_{50} values: only hemp seed oil demonstrated an antioxidant activity comparable to the control. Moreover, in the FRAP assay, consistent with the DPPH• results, the pure oils showed a minor activity respect to Vitamin E. On the contrary, the addition of CBD led to a significant enhancement in antioxidant activity surpassing that of the control. In accordance with literature, pure oils exhibit higher IC_{50} values. For instance, in Benalia and

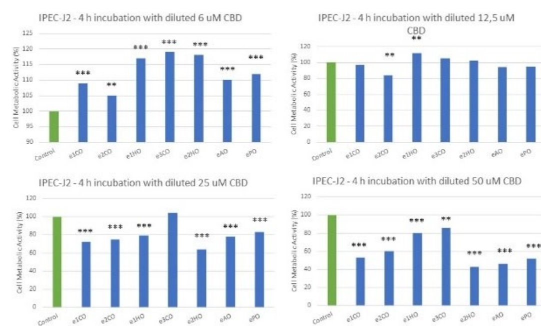

Figure 1. Results of the colorimetric MTS assay showing cell proliferation in IPEC-J2 cells treated with different concentrations of oils enriched with CBD for 4 h. The metabolic activity of cells treated with different types of oils enriched with CBD was compared with a reference control of untreated cells; results are expressed as percentages. One-way ANOVA tests: the statistical differences between each oil with respect to the reference control; ** indicates significant difference at $p<0.01$; ***$p<0.001$.

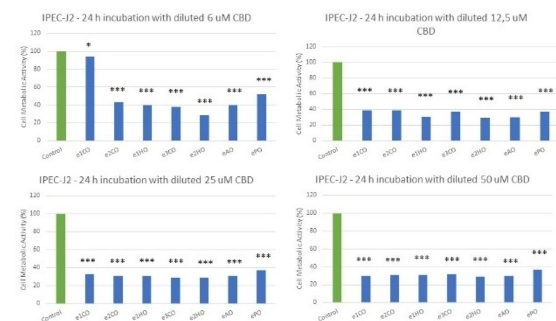

Figure 3. Results of the colorimetric MTS assay showing cell proliferation in IPEC-J2 cells treated with different concentrations of oils enriched with CBD for 24 h. The metabolic activity of cells treated with different types of oils enriched with CBD was compared with a reference control of untreated cells; results are expressed as percentages. One-way ANOVA tests: the statistical differences between each oil with respect to the reference control; * indicates significant difference at *$p<0.05$; ***$p<0.001$.

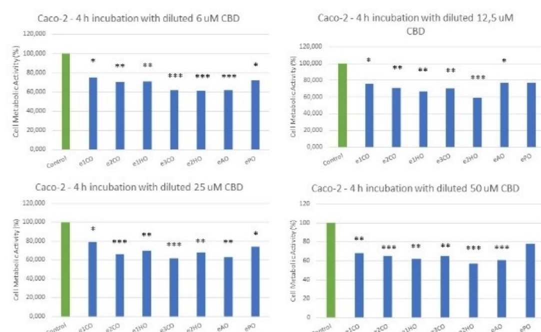

Figure 2. Results of the colorimetric MTS assay showing cell proliferation in Caco-2 cells treated with different concentrations of oils enriched with CBD for 4 h. The metabolic activity of cells treated with different types of oils enriched with CBD was compared with a reference control of untreated cells; results are expressed as percentages. One-way ANOVA tests: the statistical differences between each oil with respect to the reference control; * indicates significant difference at $p<0.05$; **$p<0.01$; ***$p<0.001$).

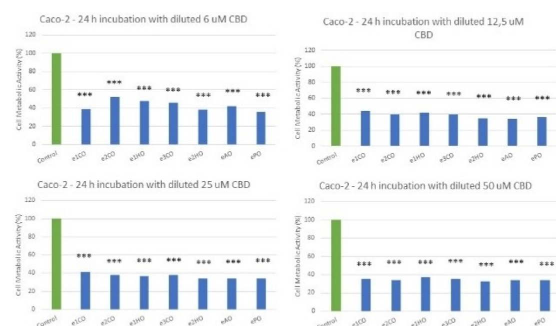

Figure 4. Results of the colorimetric MTS assay showing cell proliferation in Caco-2 cells treated with different concentrations of oils enriched with CBD for 24 h. The metabolic activity of cells treated with different types of oils enriched with CBD was compared with a reference control of untreated cells; results are expressed as percentages. One-way ANOVA tests: the statistical differences between each oil with respect to the reference control; *** indicates significant difference at $p<0.001$).

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Table 5. The effects of the pure hemp oil (pHO) and oils enriched with CBD (e1HO or e2HO) on the germination and root growth of the model organisms.

model plants	*Hordeum vulgare*			
treatment	control	pHP	e1HO	e2HO
	germinated seeds [%]			
Min	100.0	80.0	100.0	90.0
Max	100.0	100.0	100.0	100.0
Mean	100.0	93.3	100.0	96.7
Stand. dev	0.0	11.5	0.0	5.8
	root length [cm]			
Min	2.7	2.7	3.3	4.7
Max	3.8	4.9	5.8	6.3
Mean	3.2	3.6	**4.1**	**5.3a**
Stand. dev	0.6	1.2	1.4	0.9
model plants	*Triticum aestivum*			
treatment	control	pHP	e1HO	e2HO
	germinated seeds [%]			
Min	90.0	80.0	80.0	80.0
Max	100.0	100.0	90.0	100.0
Mean	96.7	90.0	86.7	90.0
Stand. dev	1.00	10.0	5.77	10.0
	root length [cm]			
Min	1.5	2.0.	3.2	2.0
Max	4.5	2.8	4.5	2.8
Mean	3.7	2.4	**3.6 A**	**2.3**
Stand. dev	1.40	0.4	0.4	0.5
model plants	*Sinapis alba*			
treatment	control	pHP	e1HO	e2HO
	germinated seeds [%]			
Min	70.0	60.0	80.0	90.0
Max	100.0	80.0	100.0	100.0
Mean	86.7	70.0	**86.7**	**93.3 A**
Stand. dev	15.28	10.0	11.55	5.77
	root length [cm]			
Min	3.2	0.7	2.6	2.9
Max	5.1	1.8	5.3	4.5
Mean	3.9	***1.4a***	**3.6**	**3.7 A**
Stand. dev	1.4	0.6	1.5	0.8
model plants	*Raphanus sativus*			
treatment	control	pHP	e1HO	e2HO
	germinated seeds [%]			
Min	20.0	20.0	20.0	30.0
Max	80.0	40.0	60.0	60.0
Mean	40.0	30.0	46.7	43.3
Stand. dev	34.6	10.0	23.9	15.3
	root length [cm]			
Min	0.6	0.4	0.7	0.8
Max	1.2	1.2	1.3	0.9
Mean	1.0	0.7	0.9	0.8
Stand. dev	0.3	0.4	0.3	0.1

Bold letters indicate the stimulation effect, bold italic and underlined letters indicate antigerminative/phytotoxic effect. The differences in germination and root growth compared to the control were evaluated using univariate statistics (T TEST). Different level of significance is indicated as follows: a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$) in comparison to water as a control; A ($p < 0.05$), B ($p < 0.01$), C ($p < 0.001$) in comparison to pure hemp oil as the control.

coworkers[31], the IC_{50} value of pumpkin seed oil was higher respect the value presented here. No studies reported the effect of pumpkin oil in the FRAP test. A recent paper[32] highlighted the antioxidant activity of argan oil as determined by FRAP assay. Another study[33] revealed that the presence of the argan oil in some formulations tested affected the results without a clear antioxidant contribution. In the $DPPH^{\bullet}$ test, the IC_{50} value of coconut oil was lower than that found here[19], while its antioxidant activity, in FRAP assay, was similar.[34] Yu et al.[35] reported that hemp seed oil presented a lower antioxidant power compared to the current study, also confirmed by FRAP assay.[36] Literature also suggests that hemp seed oil possesses a unique and probably beneficial relationship between omega-3 and omega-6 fatty acids for health: furthermore, the renewed interest for hemp seed oil was also thanks to its utilization of agri-food by-products as a novel source of proteins and natural antioxidants.[37] The same authors also reported the benefits attributed to the polyunsaturated fatty acids and cannabidiol content of the hemp plant, including anti-tumor and anti-inflammatory properties, and the promotion of treatments against oxidative stress. However, there is currently no available data on the effects of adding CBD to these oils in existing literature.

Cytotoxic Activity

The MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H- tetrazolium] assay is one of the most widely used method for assessing cytotoxicity and cell proliferation.[38] This colorimetric test is employed to measure cellular metabolic activity. This method is based on the ability of viable cells to reduce the tetrazolium compound. During incubation with MTS reagent, the dehydrogenase, enzymes within the mitochondria of healthy cells, are able to reduce the tetrazolium dyes in MTS to insoluble formazan, which can be detected at 490 nm. As cell viability decreases, dehydrogenase activity also decreases, resulting in a reduction in absorbance.[39,40]

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In this study, the cell viability was evaluated by MTS assay on commercial oils enriched with CBD, at different concentrations. The IPEC-J2 and Caco-2 cell lines were cultured in the presence of CBD oils (6, 12.5, 25, and 50 μM) for either 4 or 24 h. The cells were regularly checked under an optical microscope for morphology evaluation. No indications of cell damage were observed in any of the wells. A significant increase in absorbance was found in the non-transformed IPEC-J2 cell line, after a 4-hour incubation with six different CBD-enriched oils (e1CO, e3CO, e1HO, e2HO, eAO, ePO), at a concentration of 6 μM compared to the control ($p \leq 0.001$) (Figure 1). Similarly, the incubation with the e1HO sample, at a concentration of 12.5 μM, showed an increase in absorbance (≤ 0.01) (Figure 1). The same sample, at all other concentrations used, produced a decrease in absorbance, indicating reduced cell viability. However, the transformed Caco-2 cell lines showed a reduced absorbance after a 4-h-incubation with the lowest concentration (6 μM) of CBD-enriched oils. Comparable results were observed at the other concentrations in Caco-2 cells (Figure 2). Both cell lines, incubated with oils enriched with CBD for 24 h, showed reduced absorbance compared to the control group (cells without CBD treatment) (Figures 3 and 4). Likewise, Lowin et al.[41] found that CBD at a concentration of ≥ 5 μM, when applied to Rheumatoid Arthritis Synovial Fibroblasts (RASFs) for 6 hours, led to reduced cell viability and proliferation, suggesting that cannabidiol had the potential to reduce ongoing inflammation in conditions such as rheumatoid arthritis. Similar results were shown for different CBD oils on several cell lines with a concentration above 5 μM of CBD.[42–44] Nonetheless these studies showed that CBD had biological effects in time- and dose-dependent manner. However, in this study, when IPEC-J2 and Caco-2 cells were treated with pure oils for 4 h at the same concentrations (results not shown), no statistically significant changes were observed in cell proliferation compared to the control group. This suggests that the cell metabolic activity was influenced by the CBD. Although CBD was a non-toxic phytocannabinoid to which various anti-inflammatory effects have been attributed, this molecule was capable of binding to different enzymes and receptors, so its effect on the host is still unclear.[45]

Phytotoxic Activity

Hemp Oil

Both pure hemp oil (pHO) and CBD-enriched oils had shown no antigerminative activity on the seeds tested (Table 5). This caused a significant reduction in root growth of *Sinapis alba* ($p < 0.05$) compared to the control group. In opposite, addition of CBD, specifically when e2HO, caused significant ($p < 0.05$) stimulation of the *S. alba* germination as well as roots growth compared to pHO (Table 5), and a significant ($p < 0.05$) stimulation of *Hordeum vulgare* roots growth compared to control. Significantly ($p < 0.05$) longer roots were observed also in *Triticum aestivum* after the application of e1HO. Neither *Raphanus sativus* germination nor roots growth was affected by pHO or e1HO or e2HO.

Coconut Oil

The germination of *T. aestivum* seeds was negatively affected by the application of e2CO (coconut oil enriched with CBD), resulting in significantly ($p < 0.05$) lower germination compared to pure coconut oil (pCO). In opposite, the roots growth of *T. aestivum* was not affected by this treatment (Table 6). However, the different samples of coconut oil enriched with CBD demonstrated a significant phytotoxic effect on the root growth of *H. vulgare*, *S. alba*, and *R. sativus* (Table 6).

Argan Oil

Both pure argan oil (pAO) and argan oil enriched with CBD (eAO) did not show any significant antigerminative activity against the model organisms used (Table 7). The only phytotoxic effect was observed on *S. alba* seeds, in which the application of pAO caused significantly ($p < 0.05$) lower root growth in comparison to the water control.

Pumpkin Oil

Pure pumpkin oil (pPO) neither the pumpkin oil enriched with CBD (ePO) showed any antigerminative as well as phytotoxic activity against any of the model organisms used (Table 8). The only notable effect was observed, in *H. vulgare* seeds, where the application of ePO produced significantly ($p < 0.05$) stimulant effect compared to pPO.

Cannabis sativa EO contained a high content of CBD.[11] In the literature, a single previous study evaluated the potential herbicidal efficacy of hemp EO, exploring its impact on the germination of three crops and five weeds across varying concentrations. The findings revealed that hemp EO showed a phytotoxic potential on germination and seedling growth in weeds and also crops. The seeds of dicotyledonous displayed higher sensitivity to hemp EO compared to monocotyledonous weeds.[46] In our study, only the effects on crop seeds were evaluated: the results here reported showed that the fat hemp oil with CBD had a promoting effect on radical growth of some tested seeds. Moreover, recently, Giannini and coworkers[47] reported a potential beneficial growth effect of a coconut oil, with high level of caprylic and capric acids, on some crop species. According to Zhang et al.[48] also other fatty acids, as myristic and lauric acids, could have beneficial effects on plant biomass. Also, the phytotoxic effects of caprylic acid on weeds[49] and of middle-chain fatty acids were previously reported.[50]

The model plants used in this study have also been used in previous studies concerning the evaluation of the effects of natural compounds on germination and root elongation; indeed, recently, these crop seeds have been employed in the

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Table 6. The effects of the pure coconut oil (pCO) and the coconut oils enriched with CBD (e1CO, e2CO and e3CO) on the germination and root growth of the model organisms.

model plants	*Hordeum vulgare*				
treatment	control	pCO	e1CO	e2CO	e3CO
	germinated seeds [%]				
Min	90.0	40.0	80.0	70.0	90.0
Max	100.0	100.0	100.0	100.0	100.0
Mean	96.7	76.7	90.0	90.0	93.3
Stand. dev	5.8	32.1	10.0	17.3	5.8
	root length [cm]				
Min	2.5	2.5	4.2	2.6	2.9
Max	3.0	3.3	4.8	4.1	4.8
Mean	2.7	3.0	***4.4a, B***	3.6	3.8
Stand. dev	0.2	0.2	0.3	0.8	1.5
model plants	*Triticum aestivum*				
treatment	control	pCO	E1CO	e2CO	E3CO
	germinated seeds [%]				
Min	80.0	90.0	60.0	80.0	60.0
Max	90.0	100.0	90.0	90.0	100.0
Mean	86.7	96.7	76.7	***83.3 A***	86.7
Stand. dev	11.1	5.8	15.3	5.8	21.5
	root length [cm]				
Min	2.8	3.0	2.5	3.4	2.9
Max	4.1	4.3	3.5	4.2	4.4
Mean	3.3	3.8	3.2	3.9	3.6
Stand. dev	0.6	0.7	0.6	0.4	0.8
model plants	*Sinapis alba*				
treatment	control	pCO	e1CO	e2CO	e3CO
	germinated seeds [%]				
Min	80.0	90.0	90.0	60.0	70.0
Max	100.0	100.0	100.0	100.0	90.0
Mean	90.0	96.7	96.7	76.7	*80.0 A*
Stand. dev	10.0	5.8	5.8	20.8	10.0
	root length [cm]				
Min	3.6	3.7	3.2	4.2	2.3
Max	6.7	4.8	4.4	5.9	3.3
Mean	5.4	4.1	3.8	4.8	***2.8 A***
Stand. dev	1.6	0.6	0.6	0.9	0.5
model plants	*Raphanus sativus*				
treatment	control	pCO	e1CO	e2CO	e3CO
	germinated seeds [%]				
Min	0.0	10.0	20.0	10.0	30.0
Max	50.0	30.0	30.0	60.0	40.0
Mean	26.7	23.3	26.7	36.7	23.3
Stand. dev	25.2	11.5	5.8	25.2	15.3
	root length [cm]				
Min	2.3	0.3	1.0	0.6	0.5
Max	3.8	2.8	1.6	1.4	1.1
Mean	3.9	1.5	1.3	***0.9 a***	0.9
Stand. dev	1.7	1.2	0.3	0.4	0.3

Bold letters indicate the stimulation effect, bold italic and underlined letters indicate antigerminative/phytotoxic effect. The differences in germination and root growth compared to the control were evaluated using univariate statistics (T TEST). Different level of significance is indicated as follows: a($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$) in comparison to water as a control; A ($p < 0.05$), B ($p < 0.01$), C ($p < 0.001$) in comparison to pure coconut oil as the control.

research of selective natural herbicides, seed stimulants for cultivated plants, and inhibitors targeting weed seeds.[46] Long-chain carboxylic fatty acids are often considered prime contenders for sustainable weed control, particularly as alternatives to synthetic herbicides. These non-systemic contact herbicides have the ability to affect the cuticle and solubilize lipids.[52]

Moreover, the environmental impact of these natural products is typically minimal due to their low persistence.[52] In sight of this, also natural CBD-based compounds could be considered a starting point for future researches in the field of selective herbicides.

Conclusions

Current research has brought new insight into the application of CBD. While much of the existing literature has focused on the effects of CBD on the nervous system, the current study shows that CBD could also be used for different purposes. Its antimicrobial activity has been confirmed against specific bacterial strains, such as *P. fluorescens*, *Bacillus cereus*, *B. megaterium*, *B. mojavensis*, *X. campestris*, and *X. vesicatoria*, at 5000 mg/L or higher concentrations. Similarly, antifungal activity against *A. alternata*, *P. italicum* and *A. flavus* had also been noted. The results obtained in the two assays of antioxidant activity ($DPPH^•$ and FRAP) and on two cell lines (IPEC-J2 and Caco-2), in cytotoxic tests, let us to consider the possible use of these CBD samples as natural products with antioxidant and cytotoxic effects. The observed results after applying CBD samples to plant seeds demonstrated that, in some cases, a significant change in root growth occurred. The presented results belong to a pilot study that can be considered as starting point for further investigations.

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Table 7. The effect of the pure argan oil (pAO) and the argan oil enriched with CBD (eAO) on the germination and root growth of the model organisms.

model plants	*Hordeum vulgare*		
treatment	control	pAO	eAO
	germinated seeds [%]		
Min	100.0	90.0	80.0
Max	100.0	100.0	100.0
Mean	100.0	96.7	90.0
Stand. dev	0.0	5.8	10.0
	root length [cm]		
Min	2.8	2.4	3.6
Max	4.7	4.3	4.8
Mean	3.2	3.3	4.3
Stand. dev	0.7	0.8	0.6
model plants	*Triticum aestivum*		
treatment	control	pAO	eAO
	germinated seeds [%]		
Min	70.0	80.0	80.0
Max	100.0	90.0	100.0
Mean	83.3	83.3	90.0
Stand. dev	15.3	5.8	10.0
	root length [cm]		
Min	1.9	1.7	2.2
Max	2.5	3.5	2.7
Mean	2.2	2.5	2.4
Stand. dev	0.3	0.9	0.2
model plants	*Sinapis alba*		
treatment	control	pAO	eAO
	germinated seeds [%]		
Min	100.0	70.0	80.0
Max	100.0	100.0	100.0
Mean	100.0	83.3	93.3
Stand. dev	0.0	15.3	11.5
	root length [cm]		
Min	3.1	1.2	2.3
Max	4.3	1.8	5.3
Mean	3.5	***1.6 a***	4.0
Stand. dev	0.7	0.4	1.5
model plants	*Raphanus sativus*		
treatment	control	pAO	eAO
	germinated seeds [%]		
Min	60.0	30.0	40.0
Max	90.0	100	100.0
Mean	80.0	76.7	76.7
Stand. dev	17.1	32.1	32.1
	root length [cm]		
Min	1.9	2.4	2.3
Max	3.2	3.0	3.0
Mean	2.7	2.8	2.7
Stand. dev	0.6	0.3	0.4

Bold letters indicate the stimulation effect, bold italic and underlined letters indicate antigerminative/phytotoxic effect. The differences in germination and root growth compared to the control were evaluated using univariate statistics (T TEST). Different level of significance is indicated as follows: a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$) in comparison to water as a control; A ($p < 0.05$), B ($p < 0.01$), C ($p < 0.001$) in comparison to pure argan oil as the control.

Experimental Section

CBD Samples

For the current investigation, commercial samples of edible oils with 10% CBD from different manufacturers, available on the Slovakian market, were used. There were selected oils from different plant sources to compare the results. The oils enriched with 10% CBD were organic coconut oil (MTC), hemp seed oil, argan oil and pumpkin oil. The pure oils without CBD as organic coconut oil (MTC), hemp seed oil, argan oil and pumpkin oil were also purchased for essays to rule out the impact of the average oil. The characteristics of the pure oils and the CBD samples are listed in Table 9.

Determination of Fatty Acids Profile

Fatty acid methyl esters (FAMEs) were obtained by transmethylation following the method previously reported.[53] Chromatographic separation was performed using an HP-5MS capillary column (30 mm, 0.25 mm, and 0.25 μm) and helium as carrier gas (1 mL/min). The FAMEs injection (1 μl, 10% in *n*-hexane, v/v) was carried out in split mode (50:1). The injector temperature was 250 °C, whereas the detector temperature was 180 degrees. For GC-MS analysis, the ionization voltage, the electron multiplier, and the ion source temperature were set at 70 eV, 900 V, and 230 °C, respectively. The elution program was as following: 100 °C for 6 min, increased to 260 °C at 5 °C/min and maintained at 260 °C for 30 min. The compounds were identified by calculating their Kovats retention index with respect to the reference standard. The analyses were performed in triplicate, and the values reported were the mean ± SD.

Screening of Antimicrobial Activity

Tested Target Microorganisms

The tested bacterial strains were: *Pseudomonas fluorescens* Flügge, *Pseudomonas aeruginosa* Schröter (Migula), *Bacillus cereus* Frankland & Frankland, *Bacillus megaterium* de Bary, *Bacillus mojavensis* Roberts, *Xanthomonas campestris* Pammel, and *Xanthomonas vesicatoria* Doidge. The tested phytopathogenic fungi were *Alternaria alternata* (Fr.) Keissl., *Botrytis cinerea* Pers., *Penicillium italicum* Wehmer, and *Aspergillus flavus* Link. All tested isolates were identified by classical and molecular methods and conserved as

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Table 8. The effect of the pure pumpkin oil and the pumpkin oil with CBD on the germination and root growth of the model organism.

model plants	*Hordeum vulgare*		
treatment	control	pPO	ePO
	germinated seeds [%]		
Min	100.0	90.0	80.0
Max	100.0	100.0	100.0
Mean	100.0	96.7	90.0
Stand. dev	0.0	5.8	10.0
	root length [cm]		
Min	2.8	2.4	3.1
Max	4.7	2.9	4.3
Mean	3.2	2.6	**3.7 A**
Stand. dev	0.7	0.3	0.6
model plants	*Triticum aestivum*		
treatment	control	pPO	ePO
	germinated seeds [%]		
Min	70.0	70.0	80.0
Max	100.0	100.0	90.0
Mean	86.7	90.0	83.3
Stand. dev	15.3	17.3	5.8
	root length [cm]		
Min	1.9	1.4	1.8
Max	2.5	2.3	3.7
Mean	2.2	1.9	2.9
Stand. dev	0.3	0.5	1.0
model plants	*Sinapis alba*		
treatment	control	pPO	ePO
	germinated seeds [%]		
Min	100.0	70	80.0
Max	100.0	100	100.0
Mean	100.0	83.3	86.7
Stand. dev	0.0	15.3	11.5
	root length [cm]		
Min	2.8	2.4	3.2
Max	4.7	3.0	5.6
Mean	3.2	2.7	4.0
Stand. dev	0.7	0.3	1.0
model plants	*Raphanus sativus*		
treatment	control	pPO	ePO
	germinated seeds [%]		
Min	60.0	40.0	40.0
Max	90.0	100.0	70.0
Mean	80.0	60.0	56.7
Stand. dev	17.1	34.6	15.3
	root length [cm]		
Min	1.9	1.4	2.5
Max	3.2	2.9	3.5
Mean	2.7	2.2	3.0
Stand. dev	0.6	0.8	0.5

Bold letters indicate the stimulation effect, bold italic and underlined letters indicate antigerminative/phytotoxic effect. The differences in germination and root growth compared to the control were evaluated using univariate statistics (T TEST). Different level of significance is indicated as follows: a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$) in comparison to water as a control; A ($p < 0.05$), B ($p < 0.01$), C ($p < 0.001$) in comparison to pure pumpkin oil as the control.

pure culture in the collection of the School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Potenza, Italy.

Antibacterial Activity

The antibacterial activity was carried out following the disc diffusion method[54] using the King B (KB) nutrient media.[55] Briefly, single bacterial suspension from strain was prepared at 10^6 CFU/mL (OD≈0.2 nm) and adjusted using Turbidimetry (Biolog, Hayward, CA, USA). Four millilitres of bacterial suspension mixed with soft agar (0.7%) at ratio (9:1, v/v) were poured in each Petri dish (Ø 90 mm). Blank filter discs of 6 mm were emerged in different concentrations from the tested CBD samples (10000, 5000 and 1000 mg/L) for 30 min; then, they were placed over the KB plates. Tetracycline (160 mg/L) and Streptomycin (50 mg/L) were used as a positive controls (C+ve). The antibacterial activity was estimated by measuring the diameter of inhibition zone in mm (±SDs) eventually formed around each filter discs compared to positive control.

Antifungal Activity

The antifungal activity was evaluated against the above-mentioned pathogenic fungi following the incorporation method.[56]

For antifungal assay, the following concentrations 10000, 5000 and 1000 mg/L were used. Briefly, each sample was incorporated into Potato Dextrose Agar (PDA) nutrient medium, at 45 °C. About 0.5 cm^2 agar disc from each studied fresh fungal culture (96 h) was inoculated centrally in pre-treated PDA Petri dish (Ø 90 mm). All plates were incubated at 24 °C for 4–7 days. PDA plates without any treatments, inoculated only with each fungus, were utilized as negative control (C-ve), whereas Cycloheximide (100 mg/L) was used as the positive control (C+ve). The diameter of fungal mycelium growth was measured in mm and then the fungal inhibition percentage (F.I. %) was calculated using the following equation.[57]

$$\text{F.I. } (\%) = (\text{D.M.c- D.M.t/D.M.c}) \text{ X } 100$$

where F.I. (%): is the percentage of mycelium growth inhibition; D.M.c: is the average diameter of fungal mycelium in PDA (negative control); D.M.t: is the average diameter of fungal mycelium on treated PDA dishes.

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Table 9. List of the used CBD samples.

number	PRODUCER/DISTRIBUTER (COMPANY NAME)	CBD concentration	Dissolved in oil	Note	Identif. key
1	Argan oil	/	/	pure oil	pAO
2	Coconut oil (MTC)	/	/	pure oil	pCO
3	Pumpkin oil	/	/	pure oil	pPO
4	Hemp oil	/	/	pure oil	pHO
5	CUR.P (BS)	10%	MTC	broad spectrum	e1CO
6	CUR.P (FS)	10%	MTC	full spectrum	e2CO
7	Kon.F.L.	10%	hemp seed oil	full spectrum	e1HO
8	HEM.B.	10%	MTC		e3CO
9	Med.H.	10%	hemp seed oil		e2HO
10	KON.C (AO)	10%	argan oil	broad spectrum	eAO
11	KON.C (PO)	10%	pumpkin oil	broad spectrum	ePO

Where: p = pure, e = enriched by CBD; pAO = pure argan oil; pCO = pure coconut oil; pPO = pure pumpkin oil; pHO = pure hemp oil; e1CO = coconut oil enriched by CBD broad spectrum from the company CURE POINT; e2CO = coconut oil enriched by CBD full spectrum from the company CURE POINT; e1HO = hemp oil enriched by CBD from the company Konopná farma Liptov; e3CO = coconut oil enriched by CBD from the company HEMP BONA; e2HO = pumpkin oil enriched by CBD from the company Medi Hemp; eAO = argan oil enriched by CBD from the company KONOPE CO.; ePO = pumpkin oil enriched by CBD from the company KONOPE CO.

Antioxidant Activity

DPPH• Assay

The antiradical activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) following the protocol previously reported.[58] Aliquots of all samples were dissolved in methanol to have the final doses range from 31.25 to 1000 μg/mL. An aliquot of MeOH solution containing different amounts of the samples was added to a DPPH• solution (60 μM), to have a final volume of 1 mL in a straight-sided cuvette. An equal amount of a DPPH• solution was added in the cuvette and used as a control, while methanol alone was used as the blank. After 45 minutes, the absorbance at 515 nm was measured by a Thermo Scientific Multiskan GO spectrophotometer (Thermo Fischer Scientific, Vantaa, Finland). The results were expressed as IC_{50} value, which is the concentration necessary to reduce the absorbance of DPPH• by 50%. Tocopherol (Vitamin E) was used as standard.

FRAP Assay

FRAP (Ferric Ion Reducing Antioxidant Power) assay was performed following the protocol previously reported.[58]

The FRAP reagent, in ratio 10:1:1, was consisting of 23 mM acetate buffer (pH 3.6), 10 mM of tripyridyl triazine (TPTZ) in 400 mM of HCl and 20 mM of $FeCl_3$. Different concentrations of ferrous sulfate heptahydrate, $FeSO_4 \cdot 7H_2O$, in a range from 27.801 mg/L to 278.010 mg/L, were prepared to obtain the calibration curve (Figure 5). The reaction was carried out in well in a final volume of 272 μL. The reaction mixture was incubated in dark room at 37 °C for 30 minutes. The absorbance of the blank, consisting by FRAP alone, was subtracted from the absorbance of the FRAP with the sample. FRAP values were determined using the $FeSO_4$ $7H_2O$ calibration curve[58] and expressed as μmol Fe^{2+}/g of oil. Tocopherol (Vitamin E) was used as the reference standard.

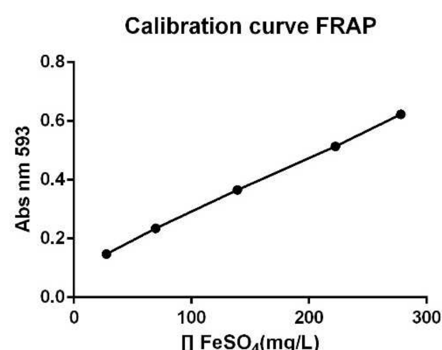

Figure 5. Calibration curve of ferrous sulfate heptahydrate.

Cytotoxic Activity

Cell lines and Culture Conditions

Both the IPEC-J2 and Caco-2 cell lines were raised from Juan José Garrido from the Department of Genetics and Animal Breeding, University of Cordoba, Spain. IPEC-J2 cells were grown and maintained in DMEM/F-12 (Sigma-Aldrich, St. Louis, MO, USA) cell media supplemented with fetal bovine serum (5% FBS; Lonza, Switzerland), glutamine (2 mM; Biosera), transferrin (10 μg/mL), insulin (10 μg/mL), selenium (10 ng/mL) (Lonza, Switzerland), epidermal growth factor (5 ng/mL; BD Biosciences, San José, CA, USA) and gentamicin (50 μg/mL; Sigma- Aldrich). Caco-2 cells were maintained in medium containing glutamine-free DMEM/Ham's F-12 (Sigma-Aldrich, USA) supplemented with fetal bovine serum (10% FBS; Lonza, Switzerland), amphotericin B (Pan Biotech, Germany) and gentamicin (5 μg/mL). Both cell lines were incubated at 37 °C in a fully humidified atmosphere with 5% CO_2, until confluency was reached. Then, the cell lines were seeded in 96-well culture plates (TPP, Switzerland) (3×10^5 cells per well) in three replicates, for 24 h cultured in DMEM/F-12 medium as mentioned above, but without supplementation (without FBS and antibiotics).

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MTS Assay

The cell viability was investigated using the Cell Titer 96 Aqueous One Solution Reagent (Promega, USA) following the manufacturer's instruction. Briefly, the both cell cultures were seeded onto a 96-well plate; after overnight cultivation, the cells became confluent and were washed with sterile phosphate buffered solution (PBS) (1X, pH 7.4). Commercial samples of each CBD oils were dissolved in 100% DMSO (Sigma-Aldrich, St. Louis, MO, USA) and then diluted for the required concentration of CBD oils in DMEM/F12 media (6; 12.5; 25; 50 μM): then, the obtained samples were added to each cell lines for 4 or 24 h. After this incubation period, the cells were washed with PBS. DMEM/F-12 media (100 μl) was added to each well after cell washing and supplemented with MTS solution (20 μl/well) incubated for 1 h at room temperature and then the absorbance was recorded at 490 nm with a 96-well micro-plate reader (Synergy HTX Multi-Mode Reader spectrophotometer, Agilent, Santa Clara, CA, USA).

Phytotoxic Activity

Solvent Preparation

Solvents were prepared for the phytotoxic activity experiment as follows: 8 mg of CBD oil or pure seed oils were placed into vials and 0.5 mL of acetone were added. Then, ultrasound bath (Bandelin Sonorex Digitec) was used for homogenization. After that, the homogenized oil was added to the 99.5 mL of distilled water.

Plant Seeds

The seeds of *Raphanus sativus* L., *Sinapis alba* L., *Triticum aestivum* L. and *Hordeum vulgare* L. were used to test phytotoxic activity. *R. sativus* and *S. alba* were purchased in local store; *T. aestivum* and *H. vulgare* were obtained from the Breeding Research Center Malý Šariš.

Phytotoxic Assay

The phytotoxic assay followed the previously used method[59] with slight modifications. Test seeds were surface sterilized in 95% EtOH for 15 seconds and rinsed triplicate in distilled water. Ten sterilized seeds were sown into each Petri dish (Ø90 mm) containing 5 layers of Whatman filter paper. In each Petri dish, 7 mL of oil solutions of different samples or distilled water/acetone 99.5:0.5 was added. Each treatment was triplicated. The Petri dishes were kept in a growth chamber (20 ± 1 °C, natural photoperiod, Sanyo, MLR-351H). Evaluation of germination and the radicle length (cm) was measured after 120 h.

Statistical Analysis

GraphPad Prism 9.0.0 and PAST[60] softwares were used to determine significant differences using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. The level of significance was set at $p \leq 0.05$ considered significant (*), $p \leq 0.01$ considered very significant (**), $p \leq 0.001$ (***) considered extremely significant, and ns considered not significant.

Author Contributions

Daniela Gruľová, Beáta Baranová and Alžbeta Glovaťáková provided samples selection and phytotoxic bioactivity. Hazem Elshafie and Ippolito Camele provided antimicrobial activity bioassays. Ľudmila Tkáčiková and Zuzana Kiššová preformed cytotoxic activity bioassays. Rosaria Francolino, Giuseppe Amato, Mara Martino, Flavio Polito, Francesco Manna performed antioxidant activity and identification of chemical composition of fatty oils. Laura De Martino, Lucia Caputo, Vincenzo De Feo managed the research activities, provided data analysis. All authors participated in preparation manuscript by writing equivalent parts.

Acknowledgements

The research was supported by the project VEGA 1/0087/20.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords: *Cannabis sativa* · biological assays · natural products · phytotoxic activity · CDB supplemented oils

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Manuscript received: February 2, 2024
Accepted manuscript online: March 11, 2024
Version of record online: ■■, ■■

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