BIOLOGICA NYSSANA
12 (2) December 2021: 113-122
DOI: 10.5281/zenodo.5759855

Antioxidant potential of commercial hemp seed oils and CBD oil

Original Article

Jelena Vitorović
Faculty of Sciences and Mathematics,
University of Niš, Višegradska 33,
18000 Niš, Serbia
jelena.rajkovic@pmf.edu.rs (corresponding author)

Nataša Joković
Faculty of Sciences and Mathematics,
University of Niš, Višegradska 33,
18000 Niš, Serbia

Andrea Žabar Popović
Faculty of Sciences and Mathematics,
University of Niš, Višegradska 33,
18000 Niš, Serbia

Abstract:

The present study investigates the antioxidant capacity of commercial hemp seed oils, 10% CBD (cannabidiol) enriched hemp seed oil and extra virgin olive oil used for comparison. Results indicate a difference in antioxidant activities among tested oils. CBD oil with IC_{50} of 0.86±0.06 mg/ml was shown to be a better scavenger of hydroxyl radical and DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical (IC_{50} value of 5.99±0.34 mg/ml) in comparison with other oils. CBD oil also had the best ability to reduce Fe^{3+} from ferricyanide at a concentration of 20 mg/ml (0.73) and the highest total antioxidant capacity (245±35.16 GAE). Two commercial fresh hemp seed oils that showed small differences in antioxidant activity had better antioxidant properties than olive oil in three performed assays, while olive oil had the highest total antioxidant capacity. Hemp seed oil showed a lower antioxidant potential after a year of storage.

Key words:
Cannabis sativa, hemp, CBD, seed oil, antioxidant

Apstrakt:

Antioksidativni potencijal komercijalnih ulja iz semena konoplje i CBD ulja

Cilj studije je ispitivanje antioksidativnog kapaciteta komercijalnih ulja dobijenih iz semena konoplje, ulja iz semena konoplje obogaćenog 10% CBD-om (kanabidiol) i ekstra devičanskog maslinovog ulja koje je korišćeno za poređenje. Dobijeni rezultati ukazuju na razliku u antioksidativnoj aktivnosti među testiranim uljima. CBD ulje koje ima IC_{50} vrednost 0.86±0.06 mg/ml se pokazalo kao bolji "hvatač" hidroksilnog radikala i DPPH (1,1-difenil-2-pikrilhidrazil) radikala (IC_{50} vrednost 5.99±0.34 mg/ml) u poređenju sa drugim uljima. CBD ulje je takođe imalo najveću sposobnost da redukuje Fe^{3+} u koncentraciji od 20 mg/ml (0.73) i najveći ukupni antioksidativni kapacitet (245±35.16 GAE). Sveža komercijalna ulja iz semena konoplje koja su pokazala male razlike u antioksidativnoj aktivnosti su imala bolje antioksidativne karakteristike od maslinovog ulja u tri korišćena testa dok je maslinovo ulje imalo najveći ukupni antioksidativni kapacitet. Ulje iz semena konoplje je pokazalo slabiji antioksidativni potencijal godinu dana nakon otvaranja.

Ključne reči:
Cannabis sativa, konoplja, CBD, ulje semena, antioksidans

Received: October 12, 2021
Revised: November 24, 2021
Accepted: November 25, 2021

Introduction

Reactive Oxygen Species (ROS) are highly reactive molecules generated normally in cells during the metabolism of oxygen. They are mainly derived from aerobic respiration during the mitochondrial electron transport but other pathways including the activity of oxidoreductase enzymes and metal catalyzed oxidation can also be involved in their production (Birben et al., 2012; Ozcan & Ogun, 2015). ROS include free radicals and radical precursors such as superoxide anion, hydroxyl radical, singlet oxygen and hydrogen peroxide. Free radicals are unstable highly reactive species because of possessing unpaired electrons that tend to pair with other molecules. At physiological concentrations, they participate in cell signaling for normal biological processes including cell proliferation, apoptosis, gene expression but when the redox balance is disturbed they can cause tissue damage by the oxidation of cellular components such as membrane lipids, proteins and DNAs (Salganik, 2001). There is a lot of evidence that ROS underly many chronic diseases including cancer, cardiovascular diseases, neurodegenerative diseases, inflammatory diseases etc. (Halliwell, 2006; Halliwell, 2007; Ferguson, 2010). The organism defends itself using endogenous cell antioxidant protection system consisted of non-

enzymatic and enzymatic components (Kurutas, 2015). It maintains balance between ROS production and removal and thus prevents organism from oxidative stress and the diseases associated with it. Antioxidants can also be exogenous, ingested via antioxidant-rich food and supplements and in this way, they can help the antioxidative defense of the cell to cope with free radicals directly by scavenging them (breaking the chain reaction) or indirectly by upregulating the antioxidant defense or inhibiting the ROS production. They act as free radicals scavengers, reducing agents, metal chelators and singlet oxygen quenchers (Lobo et al., 2010). In this way, they prevent cell damage and consequent diseases (Cox et al., 2000; Eastwood, 1999). The use of synthetic antioxidants as food preservatives or supplements has been shown to be harmful to health (Witschi, 1986). On the other hand, plants are rich source of natural antioxidants such as phenolics, flavonoids, tannins, carotenoids and vitamins with excellent antioxidant properties that have been shown in many studies. Therefore, consuming food rich in natural antioxidants is crucial for health and the development of natural products with antioxidants is of great interest to the food industry.

The plant seed oils occupy a special place in the food industry due to their nutritional value and high content of unsaturated fatty acids, as well as due to antioxidant compounds which, in addition to contributing to the stability of the oil, also have various pharmacological properties (Kriese et al., 2004). Various seed oils that have good antioxidant capacity are in use (Ramadan & Moersel, 2006; Smeriglio et al., 2016). Among them, special attention is paid to hemp seed oil (HSO), the product of the *Cannabis sativa* L. (Cannabaceae) plant, due to the specific ratio of fatty acids in its composition. The oil obtained from hemp seeds has high nutritional value and a number of health effects (Leizer et al., 2000; Callaway, 2004). It contains >80% polyunsaturated fatty acids (PUFAs) with linoleic acid as dominant, present in concentration of 52-62%, followed with α-linolenic acid in a concentrations ranging from 12-23% (Leizer et al., 2000). The linoleic acid (18:2 omega-6) and α- linolenic acid (18:3 omega-3) are present in a favorable ratio of 3:1 found to contribute to the better lipid profiles and have a cardioprotective effect (Callaway, 2004; Simopoulos, 2008; Yang et al., 2016). HSO contained also γ-linolenic acid that showed significant anti-inflammatory and immunoregulatory activity (Kapoor & Huang, 2006). The chemical analysis of the hemp seed oil also showed the presence of tocopherols, phenols, polyphenols and lignanamides which contribute to the stability of the oil and at the same time they are natural antioxidants desirable in nutrition (Kriese et al., 2004; Yan et al., 2015; Andre et al., 2016; Smeriglio et al., 2016). Tocopherols are significant in the prevention of the oxidation of PUFA in oils and have many beneficial health effects including prevention of cardiovascular diseases, cancer, and age-related macular degeneration (Kriese et al., 2004). The chemical composition of the hemp seed oil indicates its function in maintaining redox balance and lowering oxidative stress and associated diseases. *In vivo* studies have shown a beneficial effect of hemp seed oil on oxidative status and reduction of oxidative stress (Vitorović et al., 2021).

Cannabis sativa L. plant is known for the presence of compounds called cannabinoids such as delta-9 tetrahydrocannabinol (THC) and cannabidiol (CBD). Unlike THC, the most famous psychoactive cannabinoid, cannabidiol is a non-psychoactive phytocannabinoid obtained from leaves and flowers of the hemp plant that showed many pharmacological activities and therefore it is in the center of interests of the pharmacological industry. However, although preclinical and pilot studies suggest the use of CBD in the treatment of different conditions such as epilepsy, migraine, Alzheimer's disease, chronic pain, inflammatory conditions etc. (Friedman & Devinsky, 2015; Watt and Karl, 2017; Irving et al., 2018; Van Dolah et al., 2019), it is not allowed yet as a therapeutic drugs. It is already known that CBD has anti-inflammatory and antioxidant activities (Costa et al., 2007; Irving et al., 2018; Pellati et al., 2018; Atalay et al., 2020) which make it a promising agent in the prevention and treatment of illness associated with oxidative stress. CBD showed both, direct antioxidant effects through the impact on the ROS generation and components of antioxidative defense and indirect antioxidant effects, reacting with other molecules that are important for redox balance (Atalay et al., 2020).

The hemp industry become increasingly popular worldwide and many different commercial hemp products can be found in the market. The aim of the present study was to examine the possible differences in the antioxidant potential of commercial hemp seed oils from different manufacturers and to compare the effectiveness with the antioxidant capacity of CBD oil. The antioxidant activity was also compared with the well-known extra virgin olive oil, known to have oleic acid (MUFA) as a basic fatty acid in the oil (55-83%) and a lot of minor compounds (about 2% of the olive oil weight) including polyphenols, carotenoids, squalene and tocopherols (Cicerale et al., 2008; Kabaran, 2018). Since the hemp seed oil is highly rich in unsaturated fatty acids that are susceptible to oxidation which impairs their stability and function, another goal of the study was to examine how one year of storage affects the antioxidant ability of

the oil. In this case, the same commercial oil was used for the tests after the opening (HSO2) and a year later (HSO1) and the obtained results were compared.

Materials and Methods

Oil samples

In our study, we used two commercial hemp seed oils (World of hemp, Kisac and Gramina, Novi Sad), commercial CBD oil (Gramina, Novi Sad) and extra virgin olive oil (Latzimas S.A., Crete). Hemp seed oils were obtained by cold pressing while CBD oil (CBD enriched hemp seed oil) represents 10% ethanolic extract of cannabidiol dissolved in the cold pressed hemp seed oil. Oil samples used in study are listed in the **Tab. 1**. Old and New hemp seed oils represent the samples of the same oil analyzed immediately after opening (New hemp seed oil, HSO2) and one year after opening (Old hemp seed oil, HSO1). Different oil concentrations in antioxidant assays were prepared in ethanol. BHT was used as a positive control in all tests.

Table 1. Oil samples used in antioxidant tests

Samples	Abv.	Manufacturer
Old Hemp seed oil	HSO1	World of Hemp, Kisac
New Hemp seed oil	HSO2	World of Hemp, Kisac
Gramina Hemp seed oil	HSO3	Gramina, Novi Sad
Extra virgin Olive oil	EVOO	Latzimas S.A., Crete
Cannabidiol oil	CBD	Gramina, Novi Sad

In vitro antioxidant activities of oils

DPPH radical scavenging assay

DPPH assay is using to assess the reduction capacity of the antioxidants toward DPPH radical. The method is based on the scavenging of the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical by the antioxidants which change the initial purple color of DPPH solution to pale yellow due to reduction reaction. The assay was performed according to the previously described method with some modifications (Minioti & Georgiou, 2010). Hemp seed oils and olive oil in concentrations of 20, 60, 80, and 120 mg/ml and CBD oil in concentrations of 1, 5, 10, 15 and 20 mg/ml were mixed with 4 ml of DPPH solution in ethanol (0.1 mM). The mixtures were placed in the dark for 1 hour at room temperature. Discoloration of the initial purple color of DPPH solution indicates the antioxidant potential of oils, which was measured at 517 nm against ethanol blank on UV-VIS spectrophotometer (Shimadzu, 1650 PC). DPPH scavenging activity was calculated from the following formula:

$$\text{DPPH Scavenging (\%)} = ((Ab_{control} - Ab_{sample}) / Ab_{control}) * 100$$

IC_{50} which represents the concentration of the oil required to scavenge 50% of DPPH was calculated and utilized for easier comparations. The lower IC_{50} indicate the better antioxidant capacity of oils.

OH radical scavenging assay

Hydroxyl radical scavenging assay was conducted according to the previous study with some modifications (Yang et al., 2017). Hydroxyl radical was generated *in vitro* through the Fenton reaction model system. The addition of the salicylic acid (trapping agents) served to trap hydroxyl radicals because of its very short half-life leading to produce dihydroxybenzoic acids with absorption at 510 nm. Samples were dissolved in ethanol in concentrations of 0.5, 1, 2, 4 and 6 mg/ml for HSOs and Olive oil and 0.01, 0.05, 0.1, 0.5, 1 and 2 mg/ml for CBD oil. 2 ml of each oil concentration was mixed with 0.6 mL 8 mM aqueous solution of $FeSO_4$ and 2 mL 3 mM ethanol solution of salicylic acid. The reaction was activated by adding 0.5 mL of 0.6% H_2O_2, and the mixture was incubated at 37 °C for 30 min. After cooling, mixtures were centrifuged at 2000 rpm for 10 min and absorbances of supernatants were measured at 510 nm.

The hydroxyl radical scavenging activity (%) was calculated from the formula:

$$[1 - (Aa - Ac) / Ab] \times 100\%$$

where:

Aa represents the absorbance of the whole sample,

Ab represents mixture without hydrogen peroxide, with 0.5 ml dH20 ,

Ac represents blank without oil with 2 ml of ethanol.

IC_{50} was determined from the plot where % scavenging activity is presented in the function of concentration and utilized for easier comparations.

Ferric reducing power assay

The method was used according to previous studies (Baba & Malik, 2015; Bhatti et al., 2015). 0.2 ml of each oil (concentrations 5, 10, 20, 40 mg/ml for HSOs and olive oil and 0.1, 0.5, 1, 10 and 20 mg/ml for CBD oil) was added to 0.5 ml of 0.2 mM, pH 6.6 phosphate buffer and 0.5 ml of 1% potassium ferricyanide. This mixture was incubated for 20

minutes at 50 °C. Then, 0.5 ml of 10% TCA was added to the mixture followed by centrifugation at 3000 rpm for 10 minutes. 0.5 ml of supernatant was mixed with 0.5 ml of distilled water and 0.1 ml of ferric chloride (0.1%). After incubation at room temperature for 10 minutes, the absorbances were measured at 700 nm. The reducing power of the oil is represented as a function of concentration.

Phosphomolybdenum assay for total antioxidant capacity

To determine the total antioxidant capacity of the oils, the test with phosphomolybdenum was performed and the gallic acid was used as a standard. The test is based on the reduction of Mo (VI) to Mo (V) by the antioxidants and formation of a green phosphate/Mo (V) complex at acid pH. Hemp seed oils and olive oil were tested in different concentrations prepared in ethanol (5, 20, 60 and 120 mg/ml). CBD oil was tested in concentrations: 0.1, 0.5, 1, 5 and 10 mg/ml. In the test, 0.1 ml of each concentration was mixed with a previously prepared reagent composed of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate as described previously (Chahar et al., 2012). Incubation was performed at 95 °C for 90 min. The mixtures were cooled and the absorbances were measured at 695 nm. Standard curve of gallic acid prepared in concentrations of 50, 100, 150, 200 and 250 µg/ml in ethanol was used to express the total antioxidant capacity of oil as a µg equivalent of gallic acid (GAE).

Statistical analysis

All the data were expressed as means ± standard deviation (SD). The one-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison test were used for analysis. For $p<0.05$ differences were considered statistically significant.

Results and discussion

Free radical scavenging activity

Free radicals are very dangerous reactive species capable of damaging biomolecules in cells such as DNA, proteins and lipids (Salganik, 2001). Therefore, the capability to scavenge free radicals is a very important feature of antioxidants. In our study, the capability of oils to scavenge free radicals were tested in DPPH and OH scavenging assays (**Tab. 2**, **Fig. 1** and **2**).

DPPH scavenging activity

DPPH assay was conducted to estimate the antiradical activity of different oils toward DPPH radical. Results are presented in **Tab. 2** and **Fig. 1**.

CBD oil showed the best free radical scavenging activity in DPPH assay with IC_{50} of 5.99±0.34 mg/ml. The percentage of scavenging activity ranged from 32.34% to 99.69 % in the used concentrations of CBD oil (1 to 20 mg/ml). To reach 50% efficiency in scavenging DPPH radicals, it is required about ten times higher concentration of EVOO and HSO1 and about seven times higher concentration of HSO2 and HSO3 compared to CBD oil. Maximum inhibitory activity was noticed in the concentration of 120 mg/ml for all these oils and **Fig. 1a** shows that HSO2 and HSO3 showed a similar correlation between the concentration and the scavenging activity. The other two oils were less efficient scavengers until the concentration of 120 mg/ml where all the oils achieved a high degree of inhibition. This can also be seen from the calculated IC_{50} value (**Tab. 2**) which was lower for the HSO2 and HSO3 oils (39.96±1.01 and 43.09±1.21 respectively) than for HSO1 and EVOO (61.42±0.81 and 64.03±0.81, respectively). Results also indicate a lowering in the antioxidant capacity of the oil after one year of storage. The capacity of scavenging was reduced by 35% in

Table 2. Antioxidant activities of hemp seed, CBD and extra virgin olive oil

Antioxidant assays	Samples					
	HSO1	**HSO2**	**HSO3**	**EVOO**	**CBD**	**BHT**
DPPH assay (IC_{50}, mg/ml)	61.42±0.81^{b}	39.96±1.01^{d}	43.09±1.2^{c}	64.03±0.8^{a}	5.99±0.34^{e}	0.06±0.01^{f}
Phosphomolybdenum assay (GAE)	13±1.04^{b}	28.6±2.07^{b}	35.9±0.99^{b}	40.3±3.97^{b}	245±35.2^{a}	/
Hydroxyl radical scavenging assay (IC_{50}, mg/ml)	6.45±0.35^{b}	4.61±0.21^{c}	3.56±0.15^{d}	7.15±0.24^{a}	0.86±0.06^{e}	1.36±0.07^{e}

Data are presented as mean ± SD of triplicate determinations. Values in the same row with different superscript letters are significantly different ($p<0.05$). BHT was used as a positive control

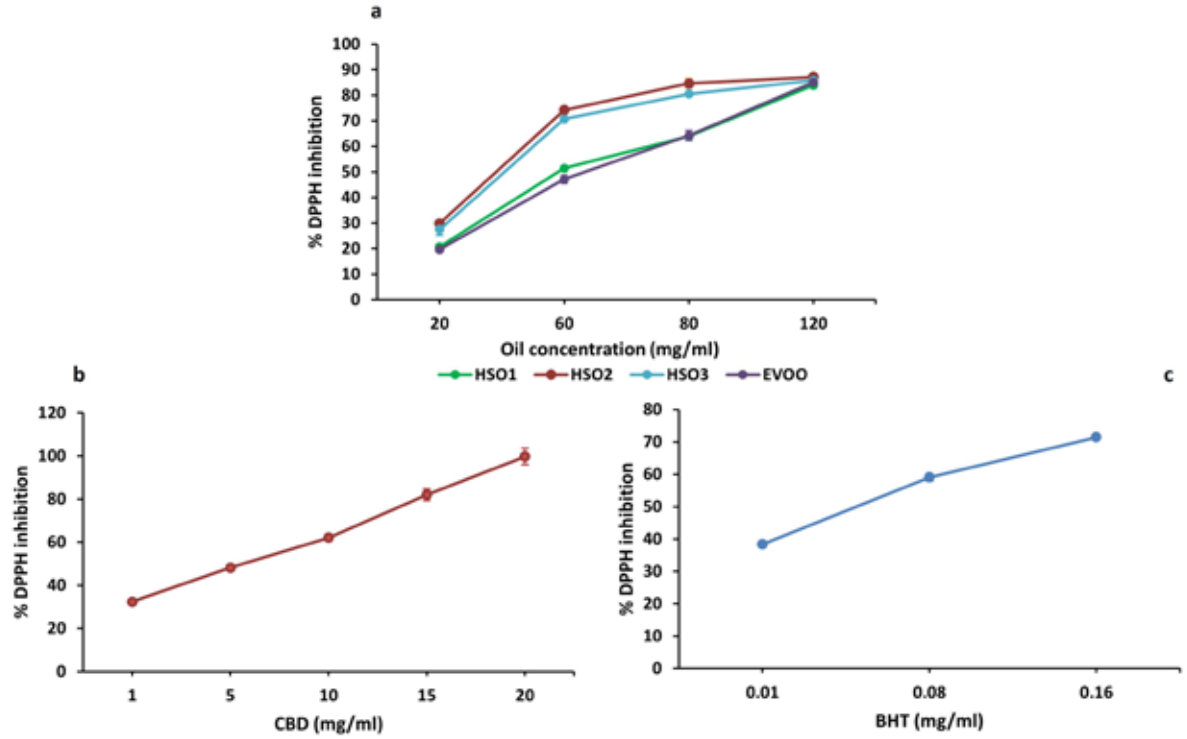

Fig. 1. DPPH scavenging activity **a)** Hemp seed oils and extra virgin olive oil, **b)** CBD oil, **c)** BHT. Data are presented as mean ± SD of triplicate determinations of the percentage scavenging of free radicals. BHT was used as a positive control

one year and, according to results this reduced oil activity was a little lower than the activity of olive oil to scavenge DPPH radical (**Tab. 2**). The most commonly used antioxidant BHT had much lower IC_{50} than all oils (0.06±0.01 mg/ml). However, it is shown that synthetic antioxidants such as BHT can cause organ damage and act as tumor promoting compounds in laboratory animals (Vuolo & Schuessler, 1985; Witschi, 1986) and that is the most important reason for using natural antioxidants, safe and effective in reducing oxidative stress.

DPPH assay as a widely used test for measuring the antiradical ability of bioactive compounds belongs to a group of tests based on both, single electron transfer (SEM) and hydrogen atom transfer (HAT) mechanisms. Studies that investigated *in vitro* antioxidant activity of seed oils showed their good scavenging activity toward DPPH radical indicating that the unsaturated fatty acids, phospholipids, phytosterols and tocopherols are carriers of this activity (Luzia et al., 2013; Cherbi et al., 2017). In the paper of Smeriglio et al. (2016), Finola HSO also showed antiradical activity and the percentage of inhibition of free radicals was the highest in the case of whole oil followed by lipophilic and hydrophilic fractions. The authors explained this activity by means of the presence of high content of flavonoids in oil that are effective in free radical scavenging and metal chelating activity

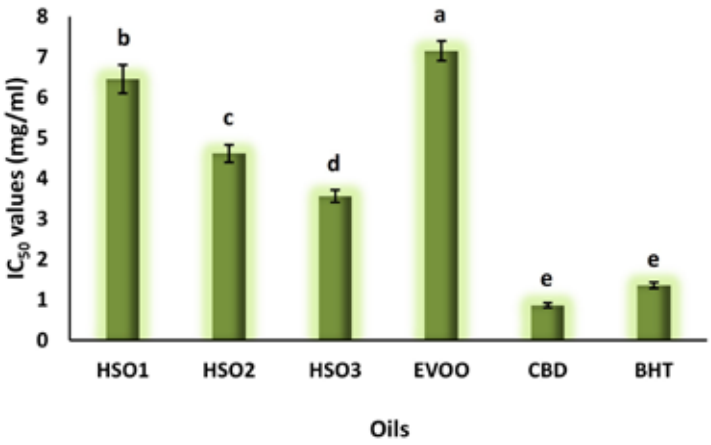

Fig. 2. Hydroxyl free radical scavenging activity presented via IC_{50} values (mg/ml) for different oils. Data are presented as mean ± SD of triplicate determinations. Values marked with different letters are significantly different ($p<0.05$). BHT was used as a positive control

(Kandaswami & Middleton, 1994). The weaker DPPH radical scavenging activity of olive oil in comparison with HSO noticed in our study was also showed in the study of Ramadan et al. (2006). They found a negative correlation between radical scavenging activity and amounts of phenolics and tocopherols and a positive correlation with levels of unsaponifiable and phytosterols among oils with the high radical scavenging activity level (coriander seed oil and black cumin seed oil) and concluded that scavenging of DPPH was combined action of different compounds in oils. It is most likely that the composition and proportion of the fatty acids and ancillary components from seed oils such are lipophilic flavonoids, tocopherol, or some other bioactive substances, are responsible for the DPPH scavenging by HSO.

In our study, CBD enriched hemp seed oil was also tested, and it was shown that CBD oil is a strong scavenger of DPPH radical which is consistent with other studies (Hacke et al., 2019; Kitamura et al., 2020; Petrovici et al., 2021). CBD is a compound with two phenolic groups in the structure and an antioxidant mechanism characteristic of phenol derivates that make it a strong antioxidant. Phenol compounds are strong DPPH scavengers by hydrogen transfer mechanism (Leopoldini et al., 2004; Leopoldini et al., 2011). Like polyphenols, CBD can eliminate free radicals via electron or hydrogen transfer from the phenol group (Borges & Da Silva, 2017).

Hydroxyl free radical (OH) scavenging activity

Hydroxyl radical is the most biologically active and dangerous free radical which can cause serious damage to biomolecules and lead to the development of disease (Lipinski, 2011; Birben et al., 2012; Ozcan & Ogun, 2015). Therefore, hydroxyl radical scavengers are very important for human's health. Generation of hydroxyl radical in Fenton reaction in the presence of hydrogen peroxide and iron ions is a model used *in vitro* to estimate hydroxyl radical scavenging by the antioxidants. For easier comparison, the IC_{50} was calculated for all tested oils and presented in **Tab. 2** and **Fig. 2**.

All the IC_{50} values are significantly different except IC_{50} for CBD and BHT indicating their similar hydroxyl radical scavenging ability. IC_{50} for the CBD oil (0.86±0.06 mg/ml) was the lowest among oils (**Fig. 2**). Moreover, all analyzed hemp seed oils had lower IC_{50} than olive oil indicating that they, together with CBD that was also dissolved in HSO, are better OH radical scavengers. Good OH radical scavenging activity of CBD oil was previously explained by the presence of two phenolic groups in cannabinoids (Borges & Da Silva, 2017; Atalay et al., 2020). Additionally, CBD is dissolved in HSO rich in PUFA that are known as good OH radical scavengers. The high affinity of hydroxyl radical to double bonds of PUFA that turn into single bounds is thought to be responsible for the scavenging activity of PUFA (Lipinski, 2011). HSO is rich in PUFA (70 to 90% of HSO are PUFA) and their content is much higher than in olive oil (Kabaran, 2018) that can explain its better OH radical scavenging activity.

The presence of polyphenolic natural substances such as flavonoids, also contributes to the antioxidant capacity of oil. Polyphenols, in addition to their ability to be oxidized, have also the ability to scavenge hydroxyl radicals reducing the double bonds to single ones and thus creating hydroxyl derivatives. This is only the case of polyphenols with available ortho position in the ring. Thus, phenol compounds that can be found in olive oil and in HSO are also responsible for their ability to scavenge OH radicals (Tuck et al., 2001). HSO2 and HSO3 were shown as excellent scavengers of OH radical especially HSO3 with the lowest IC_{50} value (3.56±0.15). The scavenging ability of HSO decreased after one year of storage which can be seen through a comparison of IC_{50} of old and new oil (6.45±0.35 for old HSO and 4.61±0.21 for the new HSO). This is probably because of the oxidation of the fatty acids in oil which impairs their anti-OH radical function.

Metal reducing ability of oils

Reduction of metals by natural compounds is another method used to evaluate the antioxidant ability (capacity) of natural compounds. For that purpose, we performed Ferric reducing power assay and Phosphomolybdenum assay.

Ferric reducing power

The ability of oils to reduce Fe^{3+} from ferricyanide into Fe^{2+} (ferrous) is mainly characteristic of antioxidants such are phenol derivatives. The reducing power of oils is presented in **Fig. 3**. Absorbance obtained at 700 nm is directly proportional to the reducing capacity of the oils. The results showed an increase in reducing potential with increasing concentration of oils. The highest reducing activity showed CBD oil at a concentration of 20 mg/ml (0.73±0.01). HSO2 and HSO3 showed maximal reducing activity of around 0.5 under the concentration of 40 mg/ml (0.51±0.01 and 0.50±0.01, respectively). Concentration of CBD oil of 0.1 mg/ml showed similar reducing power (0.24±0.004) as all other HSOs (HSO1, HSO2 and HSO3) in the concentration of 5 mg/ml (0.25±0.01, 0.24±0.003 and 0.22±0.006, respectively) as can be seen in **Fig. 3a** and **b**. CBD can reduce compounds by donating electrons and also can donate H atom to neutralize free radicals (Borges & Da Silva,

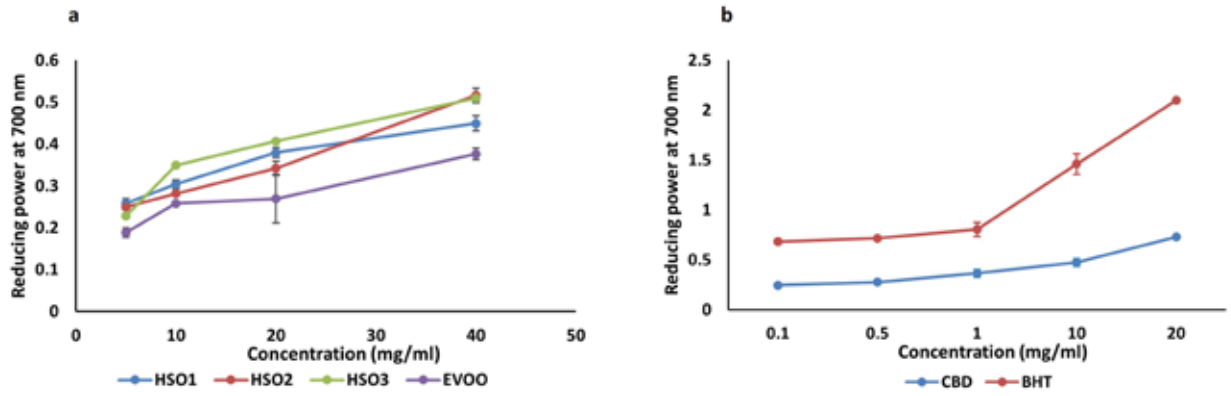

Fig. 3. Ferric reducing potential of oils **a)** Hemp seed oils (HSO) and Extra virgin olive oil (EVOO), **b)** CBD oil and BHT. Data are presented as mean ± SD of triplicate determinations. BHT was used as a positive control

2017; Atalay et al., 2020). Petrovici et al. (2021) suggested that CBD-enriched hemp oil showed high antioxidant activity having the ability to reduce iron, scavenge free radicals and inhibit lipid peroxidation. Reducing power of BHT was higher compared to all oils and the nearest to CBD oil especially in lower concentrations (**Fig. 3b**). In comparison with olive oil, all three samples of HSO in all tested concentrations showed better reducing power (**Fig. 3a**).

The results from the reducing power assay showed that the old oil (HSO1) had a very similar or even slightly higher reducing power than the new (HSO2) in lower concentrations than 40 mg/ml, which can be seen on **Fig. 3a**. A slight decrease in the reducing ability of the HSO after one year was noticed in concentration of 40 mg/ml (0.51±0.01 for the New HSO and 0.44±0.01 for the old HSO). Results that showed reducing power of the HSOs are of great importance indicating oil potential to limit the generation of free radicals via Fenton reaction. The reducing power of hemp seed extracts determined by FRAP assay was evaluated in the study of Irakli et al. (2019). It was also shown that essential oil from the aerial parts of *C. sativa* showed good reducing power (Zengin et al., 2018). Cold pressed HSO from Finola cultivar of *Cannabis sativa* reduced ferric ions, with the reducing power being better in the lipophilic fraction than in the hydrophilic fraction of oil (Smeriglio et al., 2016). Authors suggested that probably the polyphenols or tocopherol present in different amounts in HSO were mainly responsible for these reducing abilities. In the work of Petrovici et al. (2021), it was shown that reducing ability of CBD oil was in the line with the ability of phenolic derivatives.

Total antioxidant capacity

The total antioxidant capacity (TAC) of oils was estimated by Phosphomolybdenum assay. The Molybdenum assay serves to predict the antioxidant activity of the sample by measuring the reduction degree of Mo (VI) to Mo (V) by the oils and formation of green phosphate/Mo (V) complex with absorption at 695 nm. TAC of each oil was calculated as µg of gallic acid equivalent per mg of oils (GAE) using a standard gallic acid curve. In accordance with other antioxidant tests, CBD oil showed the highest total antioxidant capacity (245.00±35.16) with the statistically significant difference in comparison with all other oils (**Tab. 2**).

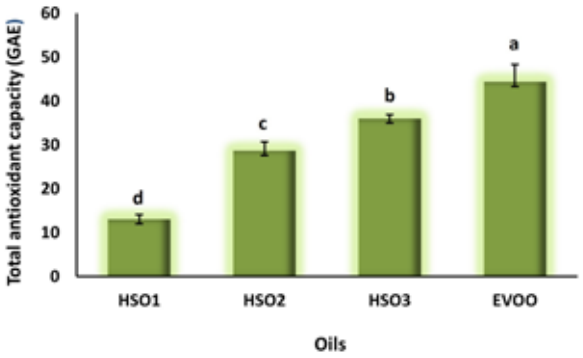

Fig. 4. Total antioxidant capacity of hemp seed oils and extra virgin olive oil. Data are presented as mean ± SD of triplicate determinations. Total antioxidant capacity is expressed as µg of gallic acid equivalent per mg of oil (GAE). Values marked with different letters are significantly different ($p<0.05$)

The results obtained from phosphomolybdenum assay for other oils than CBD (**Fig. 4**) showed that all the oil samples reduce Mo (VI) to Mo (V) with higher total antioxidant capacity obtain for EVOO (44.3±3.97 GAE) in comparison with all three samples of HSO (35.9±0.99 GAE, 28.6±2.07 GAE and 13±1.04 GAE for the HSO3, HSO2 and HSO1, respectively). This could be probably explained by the presence of the antioxidants such are phenolic

molecules and other minor compounds in olive oil since olive oil in addition to oleic acid, contains polyphenols that are known to be responsible for such antioxidant activities (Bendini et al., 2007; Lee et al., 2008). Total antioxidant activity for old HSO was 13±1.04 GAE which was more than twice less compared to fresh oil (28.6±2.07 GAE) indicating a loss of antioxidant power with storage time.

Conclusions

Oils rich in PUFA such as hemp seed oil have been the subject of various discussions regarding the impact on diseases associated with oxidative stress. *In vivo* studies have shown a beneficial effect of hemp oil on oxidative status and reduction of oxidative stress. A part of the antioxidant activities is probably due to the presence of various metabolites and antioxidants such as polyphenols or tocopherols present in the oil. The high amount of unsaturated fatty acids also indicates antioxidant activity because of the reactions that occur at the double bonds. *In vitro* antioxidant tests showed good antiradical and reduction capabilities of HSOs. Therefore, the use of hemp seed oil may be a significant contribution to the protection of diseases caused by oxidative stress but there is still a need for more *in vivo* evidence. Two commercial fresh hemp seed oils showed small differences in activity with little advantage for HSO3 noticed in the ability to scavenge OH radical and in total antioxidant capacity compared to HSO2 which showed better DPPH activity. Differences are probably due to the small variations in their composition. More *in vivo* studies are needed to examine their possible differences in the effect. Hemp seed oil didn't completely lose the antioxidant ability a year after opening although the antioxidant capacity was lower in most tests. Although the difference between CBD oil and other hemp products is often not clearly known on the market, these results indicate a difference in activity and much better antioxidant power of CBD oil which is the result of its chemical composition. In the ability to neutralize OH radicals, CBD oil has been shown to be a better antiradical scavenger even than the synthetic BHT.

Acknowledgements. This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (contract number: 451-03-9/2021-14/200124).

References

Andre, C.M., Hausman, J-F., Guerriero, G. 2016: *Cannabis sativa*: the plant of the thousand and one molecules. *Frontiers in plant science*, 7: 19.

Atalay, S., Jarocka-Karpowicz, I., Skrzydlewska, E. 2020: Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants*, 9(1): 21.

Baba, S.A., Malik, S.A. 2015: Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, 9(4): 449-454.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., Lercker, G. 2007: Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra. *Molecules*, 12(8): 1679-1719.

Bhatti, M.Z., Ali, A., Ahmad, A., Saeed, A., Malik, S.A. 2015: Antioxidant and phytochemical analysis of *Ranunculus arvensis* L. extracts. *BMC Research Notes*, 8(1): 1-8.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O. 2012: Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, 5(1): 9-19.

Borges, R., Da Silva, A. 2017: Cannabidiol as an antioxidant. In: Preedy, V. (ed), *Handbook of Cannabis and Related Pathologies*, e122-e130, Elsevier.

Callaway, J. 2004: Hempseed as a nutritional resource: An overview. *Euphytica*, 140(1): 65-72.

Chahar, M.K., Kumar, S.D., Lokesh, T., Manohara, K. 2012: Investigation of in-vitro antioxidant activity of *Mesua ferrea* L. seed oil. *International Journal of Pharmaceutical Sciences and Research*, 3(11): 4260.

Cherbi, R., Hamia, C., Gourine, N., Bombarda, I., Saïdi, M., Yousfi, M. 2017: Lipid Classes, Fatty Acids, Tocopherols Compositions and Antioxidant Activity of *Lawsonia alba* Seed Oils Growing in Algeria. *Current Nutrition and Food Science*, 13(2): 121-130.

Cicerale, S., Conlan, X.A., Sinclair, A.J., Keast, R.S. 2008: Chemistry and health of olive oil phenolics. *Critical Reviews in Food Science and Nutrition*, 49(3): 218-236.

Costa, B., Trovato, A.E., Comelli, F., Giagnoni, G., Colleoni, M. 2007: The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *European Journal of Pharmacology*, 556(1-3): 75-83.

Cox, B.D., Whichelow, M.J., Prevost, A.T. 2000: Seasonal consumption of salad vegetables and fresh fruit in relation to the development of cardiovascular disease and cancer. *Public health nutrition*, 3(1): 19-29.

Eastwood, M. 1999: Interaction of dietary antioxidants *in vivo*: how fruit and vegetables prevent disease? *QJM: An International Journal of Medicine*, 92(9): 527-530.

Ferguson, L.R. 2010: Chronic inflammation and mutagenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 690(1-2): 3-11.

Friedman, D., Devinsky, O. 2015: Cannabinoids in the treatment of epilepsy. *New England Journal of Medicine*, 373(11): 1048-1058.

Hacke, A.C.M., Lima, D., de Costa, F., Deshmukh, K., Li, N., Chow, A.M., Marques, J.A., Pereira, R.P., Kerman, K. 2019: Probing the antioxidant activity of Δ 9-tetrahydrocannabinol and cannabidiol in *Cannabis sativa* extracts. *Analyst*, 144(16): 4952-4961.

Halliwell, B. 2006: Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry*, 97(6): 1634-1658.

Halliwell, B. 2007: Oxidative stress and cancer: have we moved forward? *Biochemical Journal*, 401(1): 1-11.

Irakli, M., Tsaliki, E., Kalivas, A., Kleisiaris, F., Sarrou, E., Cook, C.M. 2019: Effect of genotype and growing year on the nutritional, phytochemical, and antioxidant properties of industrial hemp (*Cannabis sativa* L.) seeds. *Antioxidants*, 8(10): 491.

Irving, P.M., Iqbal, T., Nwokolo, C., Subramanian, S., Bloom, S., Prasad, N., Hart, A., Murray, C., Lindsay, J.O., Taylor, A. 2018: A randomized, double-blind, placebo-controlled, parallel-group, pilot study of cannabidiol-rich botanical extract in the symptomatic treatment of ulcerative colitis. *Inflammatory Bowel Diseases*, 24(4): 714-724.

Kabaran, S. 2018: Olive oil: antioxidant compounds and their potential effects over health. In Lagouri, V. (ed), *Functional Foods*, IntechOpen.

Kandaswami, C., Middleton, E. 1994: Free radical scavenging and antioxidant activity of plant flavonoids. *Free radicals in diagnostic medicine*, 351-376.

Kapoor, R., Huang, Y-S. 2006: Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. *Current Pharmaceutical Biotechnology*, 7(6): 531-534.

Kitamura, M., Kiba, Y., Suzuki, R., Tomida, N., Uwaya, A., Isami, F., Deng, S. 2020: Cannabidiol Content and *In Vitro* Biological Activities of Commercial Cannabidiol Oils and Hemp Seed Oils. *Medicines*, 7(9): 57.

Kriese, U., Schumann, E., Weber, W., Beyer, M., Brühl, L. 2004: Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa* L. genotypes. *Euphytica*, 137(3): 339-351.

Kurutas, E.B. 2015: The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, 15(1): 1-22.

Lee, O.H., Lee, B.Y., Kim, Y.C., Shetty, K., Kim, YC. 2008: Radical scavenging-linked antioxidant activity of ethanolic extracts of diverse types of extra virgin olive oils. *Journal of Food Science and Technology*, 73(7): C519-C525.

Leizer, C., Ribnicky, D., Poulev, A., Dushenkov, S., Raskin, I. 2000: The composition of hemp seed oil and its potential as an important source of nutrition. *Journal of Nutraceuticals, functional and medical foods*, 2(4): 35-53.

Leopoldini, M., Marino, T., Russo, N., Toscano, M. 2004: Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. *The Journal of Physical Chemistry A*, 108(22): 4916-4922.

Leopoldini, M., Russo, N., Toscano, M. 2011: The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry*, 125(2): 288-306.

Lipinski, B. 2011: Hydroxyl radical and its scavengers in health and disease. *Oxidative Medicine and Cellular Longevity*, 2011.

Lobo, V., Patil, A., Phatak, A., Chandra, N. 2010: Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8): 118.

Luzia, D.M., Jorge, N. 2013: Bioactive substance contents and antioxidant capacity of the lipid fraction of *Annona crassiflora* Mart. seeds. *Industrial Crops and Products*, 42: 231-235.

Minioti, K.S., Georgiou, C.A. 2010: Comparison of different tests used in mapping the Greek virgin olive oil production for the determination of its total antioxidant capacity. *Grasas y aceites*, 61(1): 45-51.

Ozcan, A., Ogun, M. 2015: Biochemistry of reactive

oxygen and nitrogen species. *Basic principles and clinical significance of oxidative stress*, 3: 37-58.

Pellati, F., Borgonetti, V., Brighenti, V., Biagi, M., Benvenuti, S., Corsi, L. 2018: *Cannabis sativa* L. and nonpsychoactive cannabinoids: their chemistry and role against oxidative stress, inflammation, and cancer. *BioMed research international*, 2018.

Petrovici, A.R., Simionescu, N., Sandu, AI., Paraschiv, V., Silion, M., Pinteala, M. 2021: New Insights on Hemp Oil Enriched in Cannabidiol: Decarboxylation, Antioxidant Properties and *In Vitro* Anticancer Effect. *Antioxidants*, 10(5): 738.

Ramadan, M.F., Moersel, J-T. 2006: Screening of the antiradical action of vegetable oils. *Journal of Food Composition and Analysis*, 19(8): 838-842.

Salganik, R.I. 2001: The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *Journal of the American College of Nutrition*, 20(5): 464S-472S.

Simopoulos, A.P. 2008: The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental biology and medicine*, 233(6): 674-688.

Smeriglio, A., Galati, E.M., Monforte, M.T., Lanuzza, F., D'Angelo, V., Circosta, C. 2016: Polyphenolic Compounds and Antioxidant Activity of Cold-Pressed Seed Oil from Finola Cultivar of *Cannabis sativa* L. *Phytotherapy Research*, 30(8): 1298-1307.

Tuck, K.L., Freeman, M.P., Hayball, P.J., Stretch, G.L., Stupans, I. 2001: The *in vivo* fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *The Journal of nutrition*, 131(7): 1993-1996.

Van Dolah, H.J., Bauer, B.A., Mauck, K.F. 2019: *Clinicians' guide to cannabidiol and hemp oils*. Mayo Clinic Proceedings, Elsevier, 1840-1851 p.

Vitorović, J., Joković, N., Radulović, N., Mihajilov-Krstev, T., Cvetković, V.J., Jovanović, N., Mitrović, T., Aleksić, A., Stanković, N., Bernstein, N. 2021: Antioxidant Activity of Hemp (*Cannabis sativa* L.) Seed Oil in *Drosophila melanogaster* Larvae under Non-Stress and H_2O_2-Induced Oxidative Stress Conditions. *Antioxidants*, 10(6): 830.

Vuolo, L., Schuessler, G. 1985: Review: Putative mutagens and carcinogens in foods. *Environmental Mutagenesis*, 7: 577-598.

Watt, G., Karl, T. 2017: *In vivo* evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's disease. *Frontiers in Pharmacology*, 8: 20.

Witschi, H. 1986: Enhanced tumour development by butylated hydroxytoluene (BHT) in the liver, lung and gastro-intestinal tract. *Food and Chemical Toxicology*, 24(10-11): 1127-1130.

Yan, X., Tang, J., dos Santos Passos, C., Nurisso, A., Simoes-Pires, C.A., Ji, M., Lou, H., Fan, P. 2015: Characterization of lignanamides from hemp (*Cannabis sativa* L.) seed and their antioxidant and acetylcholinesterase inhibitory activities. *Journal of Agricultural and Food Chemistry*, 63(49): 10611-10619.

Yang, L.G., Song, Z.X., Yin, H., Wang, Y.Y., Shu, G.F., Lu, H.X., Wang, S.K., Sun, G.J. 2016: Low n-6/n-3 PUFA ratio improves lipid metabolism, inflammation, oxidative stress and endothelial function in rats using plant oils as n-3 fatty acid source. *Lipids*, 51(1): 49-59.

Yang, X., Zhang, D., Song, L-m., Xu, Q., Li, H., Xu, H. 2017: Chemical profile and antioxidant activity of the oil from Peony seeds (*Paeonia suffruticosa* Andr.). *Oxidative Medicine and Cellular Longevity*, 2017.

Zengin, G., Menghini, L., Di Sotto, A., Mancinelli, R., Sisto, F., Carradori, S., Cesa, S., Fraschetti, C., Filippi, A.,Angiolella, L. 2018: Chromatographic analyses, *in vitro* biological activities, and cytotoxicity of *Cannabis sativa* L. essential oil: A multidisciplinary study. *Molecules*, 23(12): 3266.