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ORIGINAL SCIENTIFIC PAPER

Antioxidant and antimicrobial potential of essential oils of different types of pepper (*Piper sp.*)

Antioksidativni i antimikrobni potencijal eteričnih ulja različitih vrsta bibera (*Piper sp.*)

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ABSTRACT

Essential oils are mixtures of potent compounds with biological effects, such as those with antioxidant, antimicrobial, antitumor and/or anti-inflammatory effects. In this paper, the antioxidant and antimicrobial activity of essential oils of different types of commercial pepper was tested. Essential oils are prepared by hydrodistillation. Polyphenol content, reduction potential and DPPH radical inhibition were analyzed. Antimicrobial activity was tested on reference strains using the diffusion technique. The analysis showed that the essential oil of black pepper has a significantly higher antioxidant potential compared to the essential oils of green and white pepper. High antimicrobial activity was also found for the black pepper essential oil sample, except in the case of *Pseudomonas aeruginosa*.

Key words: *black pepper, white pepper, green pepper, FRAP, DPPH*

INTRODUCTION - *Uvod*

Piper nigrum L. is an aromatic plant that belongs to the Piperaceae family. This plant is cultivated in tropical regions such as central and northern South America, and the Asia Pacific region. Since prehistoric times *Piper* sp. have been used by humans mainly as spices and in folk medicine to treat many diseases. Due to diverse biological activities, mainly anti-inflammatory and analgesic properties, many species of *Piper* have the potential for use in the pharmaceutical industry (Dyer et al., 2004). Essential oils are complex mixtures of volatile substances that are lipophilic. They can contain different compounds at various concentrations. The composition of essential oils is not constant and varies according to genotype, environmental factors, geographical origin, and plant cultivation and collection procedures (Bakkali et al., 2008). The pepper essential oils are characterized by the presence of monoterpenes, sesquiterpenes and phenylpropanoids with significant biological effects (Parmar et al., 1997). Essential oils from pepper are an important source of substances that have biological properties such as antioxidant, antimicrobial, anti-inflammatory and antifungal activities (Silva et al., 2020, Chahal et al., 2011). The antioxidant activity of essential oils of different types of pepper has been confirmed

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through several studies (Wang et al., 2021; Zhang and Xu, 2015). The aim of this study was to evaluate the antioxidant and antimicrobial activity of the essential oils obtained by hydrodistillation of three commercially available pepper samples.

MATERIALS AND METHODS – *Materijal i metode*

All pepper seed samples were commercially available. White pepper originates from India, while green and black pepper originate from Brazil. Demineralized water was used to prepare the solution for spectroscopic measurements. Folin-Ciocalteu reagent for testing polyphenol content and sodium carbonate were purchased from Semikem, Bosnia and Herzegovina. Methanol HPLC grade, 2,4,6-Tripyndyl-s-triazine, iron(III) chloride, hydrochloric acid, sodium acetate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Spectroscopic measurements were performed on a Perkin Elmer λ25 spectrophotometer.

Hydrodistillation

Five hundred grams of finely ground pepper samples were subjected to hydrodistillation using Clevenger-type apparatus for 5 h according to the European Pharmacopoeia. Essential oils were dried over sodium sulfate anhydrous and the sample was stored at 4°C before use.

Determination of total phenolic content (TPC)

Total phenolic compounds present in the essential oil were quantified spectrophotometrically through the Folin-Ciocalteu test following the protocol (Singleton et al., 1999). 200 µL of essential oil was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 µL of 10% sodium carbonate was added. 910 µL distilled water was added to each sample prior to measuring. The absorbance of the resulting blue-coloured solution was measured at 765 nm.

Examination of the reducing ability (FRAP method)

The test of the reducing ability of the essential oil was tested using the FRAP (ferric reducing antioxidant power) method, according to the published procedure (Benzie and Strain, 1999). 3 mL of prepared FRAP reagent was mixed with 100 µL of essential oil. Absorbance was measured at 593 nm after 30 min incubation at 37°C.

Inhibition of DPPH radicals

A DPPH radical inhibition assay was performed according to a published method (Horozić et al., 2019). Essential oils were mixed with absolute methanol and then mixed with DPPH radical solution. Absorbance measurements were performed at 517 nm, after which DPPH radical inhibition was calculated according to the equation:

$$I = \frac{Ac - As}{Ac} x100$$

where As is the absorbance of the solution containing the sample at 517 nm, and Ac is the absorbance of the DPPH solution.

Analysis of antimicrobial activity

The *in vitro* antibacterial activities of the essential oils were investigated using Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). *C. albicans* was used to test the antifungal activity of the isolated oils. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 10^7-10^8 CFU/mL). The strains were then placed on the surface of the nutrient substrate Mueller-Hinton agar, and dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar, sterile drill-shaped holes were made ("wells"), into which 100 µL of essential oil was poured. After the plates were left at room temperature for 15 minutes,

Table 2. Polyphenol content and antioxidant activity of pepper essential oils

Tabela 2. Sadržaj polifenola i antioksidativna aktivnost eteričnih ulja bibera

Samples	TPC [mg GAE/g]	FRAP [µmol/g]	IC_{50} [mg/mL]
Black pepper	1.21	86.16	7.67
Green pepper	0.40	25.6	40.86
White pepper	0.94	28.8	45.02
Vitamin C	-	14 250	0.03

the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured.

RESULTS - *Rezultati*

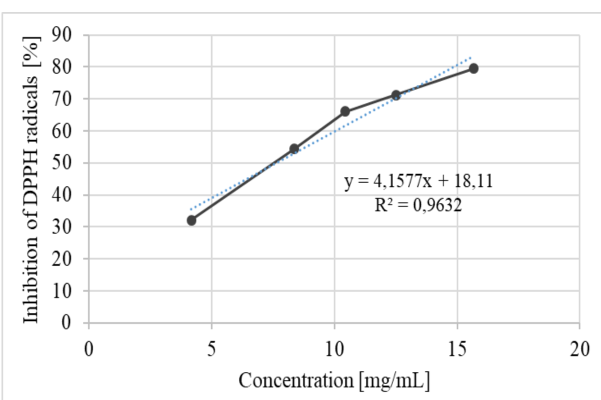
Hydrodistillation obtained essential oils with a particularly strong aroma, with a good yield. Table 1 shows the yield of isolated oil depending on the type of pepper.

Table 1. Yields of isolated pepper essential oils

Tabela 1. Prinosi izoliranih eteričnih ulja bibera

Essential oil	Yield [%]
Black pepper	1.34
Green pepper	0.47
White pepper	1.77

Table 2 shows the results of the content of polyphenols, as well as the values of reduction potential and DPPH radical inhibition in *in vitro* conditions. Vitamin C was used as a reference. Figure 1 shows graphs of the dependence of DPPH radical inhibition on concentration, through which the IC50 value for essential oil samples was calculated.



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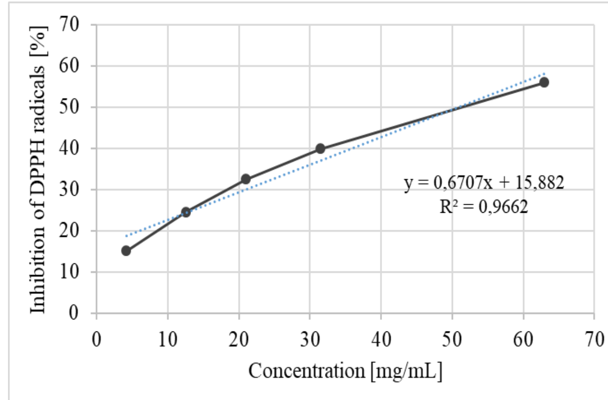

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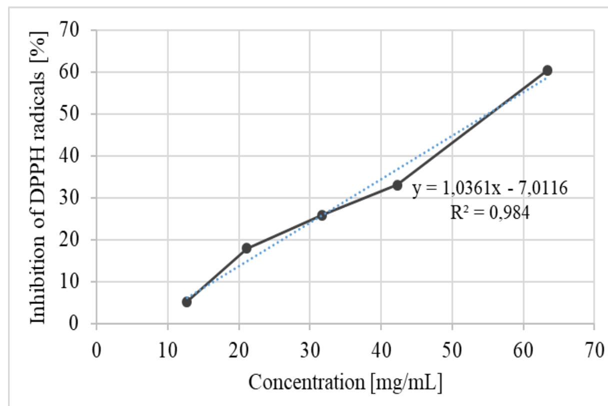

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Graph 1. Representation of the dependence of oil concentration on the percentage of quenching of DPPH radicals: (A) black pepper, (B) green pepper and (C) white pepper

Grafikon 1. Prikaz ovisnosti koncentracije ulja od procenta gašenja DPPH radikala (A) crnog bibera, (B) zelenog bibera i (C) bijelog bibera

Table 3 shows the results of testing antimicrobial activity in *in vitro* conditions.

Table 3. Antimicrobial activity of pepper essential oils

Tabela 3. Antimikrobna aktivnost eteričnih ulja bibera

Essential oil	Inhibition zone [mm]				
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
Black pepper	10	NA	15	15	13
Green pepper	9	NA	12	11	9
White pepper	NA	NA	8	NA	9

*NA - No activity

DISCUSSION – *Diskusija*

The content of polyphenols in essential oils of commercial peppers is relatively low, and is correlated with antioxidant capacity. The highest total phenol content was measured in the black pepper sample which also showed the highest antioxidant potential. Li et al. (2020) recently studied black and white pepper and they found that black pepper fruit was slightly better than white pepper fruit in terms of antioxidant activity. From Table 2 it can be seen that the effective concentration (EC50) value of the black pepper fruit was 7.67 mg/mL, which is in good agreement with the previous study (Wang et al., 2021). However, these values obtained for white pepper are significantly lower than those reported previously (Zhang and Xu, 2015). The DPPH radical neutralization efficiency as well as the reduction potential of all tested essential oils is significantly lower than the values obtained for vitamin C, well known antioxidant which was used as a control.

Pepper essential oils generally showed a better antimicrobial effect against gram-positive bacteria *B. subtilis* and *S. aureus*, with an inhibition zone in the range of 8-15 mm. The highest sensitivity was confirmed with the essential oil of black and green pepper. White pepper essential oil showed extremely weak antimicrobial activity in *in vitro* conditions. All tested samples showed antifungal activity. Through research conducted in the last few years, the antibacterial effect of the essential oil of black (Morsy and El-Salam, 2017; Hikal, 2018), white (Singh et al., 2013) and green pepper (Myszka et al., 2019) has been confirmed. In the aforementioned studies, larger zones of inhibition were recorded, which can be explained by the different origin of the samples, and the way the samples were treated and stored.

The presence of terpenoids in pepper essential oil is responsible for its antimicrobial potential (Menon et al., 2003). An important characteristic of essential oil components is their hydrophobicity, which enables them to partition into the lipids of the bacterial cell membrane, disturbing the cell structures, rendering them more permeable, and leading to lysis and leakage of intracellular compounds (Gill and Holley, 2006; Bajpai et al., 2013).

This study on the antioxidant and antimicrobial activities of essential oil from black, green and white pepper fruit proves that pepper essential oils exhibit antioxidant and antimicrobial effects owing to their active components. Black pepper essential oil was more effective than white and green pepper essential oil which is caused by differences in essential oil compositions. From the result of this study, it can be concluded that pepper essential oils have great potential as a natural source of therapeutic agent for bacterial infection. Further studies regarding the chemical composition of essential oils should be performed.

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SAŽETAK

Eterična ulja su mješavine potentnih spojeva s biološkim djelovanjem, poput onih s antioksidativnim, antimikrobnim, antitumorskim i/ili protuupalnim djelovanjem. U ovom radu ispitana je antioksidativna i antimikrobna aktivnost eteričnih ulja različitih vrsta komercijalnog bibera. Eterična ulja su pripremljena hidrodestilacijom. Analiziran je sadržaj polifenola, redukcijski potencijal ulja i inhibicija DPPH radikala. Antimikrobna aktivnost ispitana je difuzionom tehnikom na referentnim sojevima. Analiza je pokazala da eterično ulje crnog bibera ima značajno veći antioksidativni potencijal u odnosu na eterična ulja zelenog i bijelog bibera. Za uzorak eteričnog ulja crnog bibera također je utvrđena visoka antimikrobna aktivnost, osim u slučaju *Pseudomonas aeruginosa*.

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