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ORIGINAL SCIENTIFIC PAPER

In vitro antioxidant activity of methanolic extracts of the different types of commercial pepper

In vitro antioksidativna aktivnost metanolnih ekstrakata različitih vrsta komercijalnog bibera

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ABSTRACT

Pepper is one of the most famous and widespread spices in the world. This commercial plant belongs to the Piperaceae family. Depending on the treatment to which the pepper fruit is exposed, we distinguish between black, white and green pepper. In this research, methanolic extracts of different types of pepper (*Piper nigrum*) and pink pepper (*Schinus terebinthifolius Raddi*) were prepared. Maceration and ultrasonic extraction were used to extract bioactive components from pepper samples. The reduction potential of the extracts was tested using the FRAP method. The efficiency of free radical inhibition was determined by the DPPH method. Pink pepper extract showed the highest antioxidant activity in *in vitro* conditions. High antioxidant activity was also recorded in green pepper extracts. In general, the extracts showed high potency in neutralizing free radicals.

Key words: *Black pepper, Green pepper, White pepper, Pink pepper, Polyphenols, FRAP, DPPH*

INTRODUCTION – *Uvod*

Many plants, i.e. their parts such as fruits, leaves, flowers or roots, have been used since ancient times for the preparation of traditional medicines. Many of them are also consumed as teas or spices that contribute to improving the taste, smell and color of food (Zarai et al., 2013; Dinesha and Chikkanna, 2014). Pepper (*Piper nigrum*) is one of the most common and popular spices. This climbing plant belongs to the Piperaceae family. It is grown mainly in tropical regions, such as western and southwestern India, Malaysia, Indonesia, and Brazil (Mathew et al., 2001; Srinivasan, 2007; Zahin et al., 2021).

The dominant component of pepper is piperine ((2E,4E)-5-(2H-1,3-benzodioxol-5-yl)-1-(piperidin-1-yl) penta-2,4-dien-1-one), a compound of monoclinic crystals that is responsible for the sharpness of the pepper's taste. It is an integral part of pharmaceutical preparations because it has been found to improve their bioavailability (Tripathi et al., 1996; Khajuria et al., 2002). Other compounds that are present in smaller amounts in pepper are: safrole, sabinene, pinene, limonene, linalool and caryophyllene (Khan et al., 2021). Fi-

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gure 1 shows the structures of the dominant compounds contained in pepper.Various parts of pepper, including secondary metabolites are used as medicines, preservatives, insecticidal and larvicidal agents (Ahmad et al., 2012). According to the available literature and conducted research, pepper exhibits various biological activities, which have been proven through *in vitro* and *in vivo* studies of extracts and essential oils: antitumor (Paarakh et al., 2015; Reddy et al., 2015; Zhaomei et al., 2008; Dayem et al., 2016), antimicrobial (Kavitha and Mani, 2017; Rani and Saxena, 2013; Pradhan et al., 1999; Karsha and Lakshmi, 2010; Zhang et al., 2017), hepatoprotective (Zhang et al., 2021) and antidepressant activity (Hritcu et al., 2015). The research conducted by Tiwari and Singh found that the components present in pepper can stimulate the digestive enzymes of the pancreas and intestines and increase the secretion of bile acids (Tiwari et al., 2008). Piperine has been identified as a positive allosteric modulator of the □-aminobutyric acid type A benzodiazepine receptor in *in vitro* cell models (Khom et al., 2013; Zaugg et al., 2010).

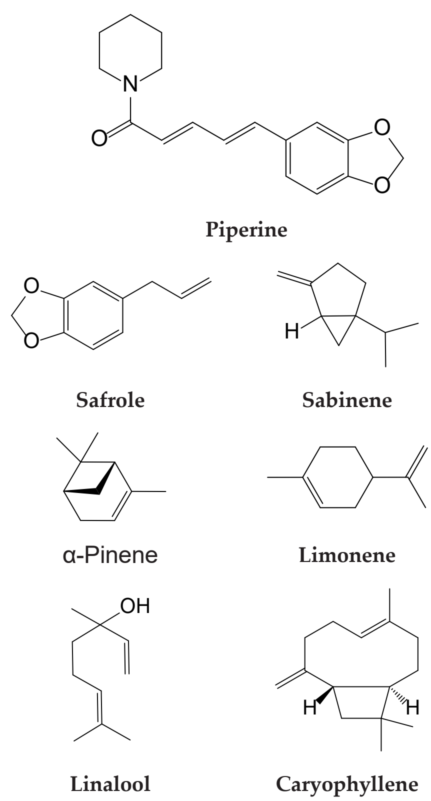

Figure 1. Structures of dominant compounds of pepper

Slika 1. Strukture dominantnih komponenti bibera

Depending on the processing method, there are several types of pepper commercially available, of which black, green and white pepper stand out. Black pepper is obtained by boiling small peppercorns while they are still green, after which they are dried and turn black. White pepper is produced by removing the peel and flesh of the fruit, leaving only the seed or grain. This is achieved by soaking the fruit in water for several days. Green pepper is most often obtained like black pepper, with the fact that sulfur dioxide is used to treat the fruit, which gives the grain green color. Pink pepper (*Schinus terebinthifolius Raddi*) does not belong to the pepper family but originates from South America and belongs to the cashew family. It has a pronounced fruity taste, does not have the intense spiciness of real pepper, and has a more delicate taste.

The aim of this paper is to examine the influence of the extraction technique on the antioxidant activity of methanolic extracts of different types of pepper and to compare their antioxidant potential.

MATERIALS AND METHODS – *Materijali i metode*

Plant material and chemical agents

The pepper samples were bought in a local market in Tuzla. White pepper (1) originates from India, pink pepper (2) from Vietnam, while green (3) and black (4) pepper originate from Brazil. Demineralized water was used to prepare the solution for spectroscopic measurements. Methanol used for extraction and DPPH radical neutralization assay was of HPLC grade. Folin-Ciocalteu reagent for testing polyphenol content and sodium carbonate were purchased from Semikem, Bosnia and Herzegovina. 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 □25 spectrophotometer.

Preparation of extracts

Pepper samples were ground in an electric mill (Končar MK45) before extraction. 8 grams of crushed pepper was transferred to a 100 mL Erlenmeyer flask and poured over with 80 mL of methanol. An ultrasonic bath ElmaSonic S was used to prepare the first batch of extracts. A Vibromix 40 was used to prepare the extracts by maceration at room temperature at 300 rpm. After an hour, the mixtures were filtered through filter paper. The extracts were immediately subjected to analysis. In the rest of the text and when presenting the results, the extracts will be marked with numbers as stated before, with the mark M for extracts prepared by maceration and the mark UE for extracts prepared using ultrasonic extraction.

Determination of total phenolic content (TPC)

Total phenolic compounds present in the extracts were quantified spectrophotometrically through the Folin-Ciocalteu test following the protocol (Singleton et al., 1999). 200 µL of extracts 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-colored solution was measured at 765 nm.

Examination of the reducing ability of the extract

The test of the reducing ability of the pepper extract 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 extracts. Absorbance at 593 nm was recorded 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). Extracts were mixed with absolute methanol and then mixed with DPPH radical solution. Absorbance measurements were performed at 517 nm. The results are expressed as a percentage of DPPH radical inhibition for the used extract concentration of 0.5 mg/mL.

RESULTS AND DISCUSSION - *Rezultati i diskusija*

The results of the analysis of the content of polyphenols in the methanol extracts of different types of pepper are shown in Table 1. The highest content of polyphenols was recorded in the extracts of pink pepper, then in the extract of green pepper, while the lowest content of these components was recorded in the methanol extract of white pepper.

Table 1. Content of total polyphenols in pepper extracts

Tabela 1. Sadržaj ukupnih polifenola u ekstraktima bibera

Sample	TPC [mg GAE/g]
M-1	0.15
UE-1	0.28
M-2	1.73
UE-2	2.01
M-3	1.53
UE-3	1.60
M-4	0.81
UE-4	1.12

The reason for the significant difference in the content of polyphenols in these extractions, in addition to the different geographical origin of the samples, may also be the storage methods of the samples, their age, as well as the method of processing the sample with the aim of obtaining the final product (exposure to high temperatures, cooking, and exposure to certain gases during processing). Of all the pepper samples, according to the literature, pink pepper does not undergo excessive processing of the sample before being put on sale. Namely, pink peppercorns are simply dried, without cooking.

Tables 2. and 3. show the results of the reducing ability and DPPH radical inhibition efficiency of methanol extracts of pepper. DPPH radical inhibition values and reduction potential of methanolic extracts are correlated with polyphenol content. The highest antioxidant capacity was found in the extracts of pink and green pepper. Ultrasonic extraction is more effective in extracting bioactive components responsible for antioxidant activity.

Table 2. Reducing ability of pepper extracts

Tabela 2. Redukcijska sposobnost ekstrakata bibera

Sample	FRAP value [µmol/g]
M-1	94.62
UE-1	100.22
M-2	102.65
UE-2	222.04
M-3	91.44
UE-3	108.31
M-4	81.15
UE-4	86.91

Table 3. DPPH radical inhibition efficiency

Tabela 3. Efikasnost inhibicije DPPH radikala

Sample	DPPH inhibition [%]
M-1	16.63
UE-1	16.63
M-2	92.43
UE-2	93.06
M-3	25.53
UE-3	30.42
M-4	20.64
UE-4	22.06

In the last few years, the antioxidant activity of essential oils and extracts of different types of pepper has been analyzed, with special reference to black pepper, for which there are the most results. Dae Won Kim et al. (2020) compared the physicochemical properties and antioxidant capacity of commercial black and green pepper. The extracts were prepared in 80% ethanol while stirring for 24 hours. The extracts showed a significantly higher content of polyphenols and antioxidant capacity. Sruthi et al. (2017) examined the antioxidant and cytotoxic activity of selected black pepper samples. Solvents of different polarity were used to prepare the extracts: n-hexane, chloroform, methanol and water, and Soxhlet extraction was used as the extraction technique. Chloroform and methanol extracted the most polyphenols. Aqueous extracts contained the least polyphenols, which was also reflected in the antioxidant activity. Lower values of polyphenols are expected for aqueous extracts obtained by Soxhlet extraction, as this technique is rarely used in cases where water is the solvent. The reason for this is the slower extraction flow, denser extracts and turbidity of the extract. Menegali et al. (2020) examined the antioxidant activity of pink pepper extracts prepared by a combination of maceration at elevated temperature and ultrasonic extraction. The obtained results of polyphenol content and antioxidant activity are higher compared to our research. Higher values of polyphenol content, i.e. antioxidant activity of red pepper extracts were reported through other, similar studies (Merlo et al., 2019; Serrano-León et al., 2018; Romani et al., 2018).

CONCLUSIONS – *Zaključak*

The results of the analyzes carried out through this research show that pepper extracts possess antioxidant capacity and can be used in the inhibition of free radicals. Pink pepper extract showed a far better antioxidant effect than other types of pepper. Ultrasound-assisted extraction is more effective in extracting bioactive components from pepper samples, compared to maceration. For a more detailed insight into the efficiency of the extraction of bioactive components from pepper, it is necessary to analyze the influence of other solvents, which will certainly be the subject of research in the coming period.

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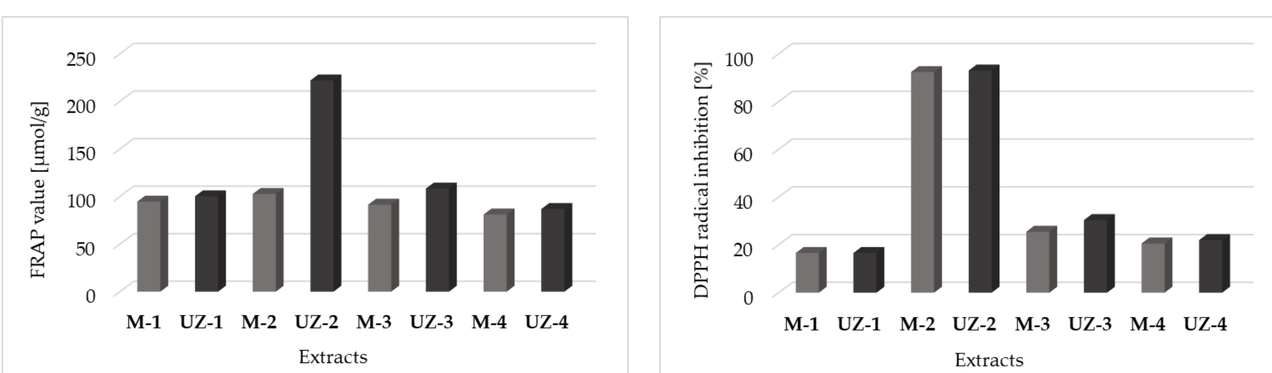

Figure 2. Comparison of antioxidant activity of methanol extracts of pepper

Slika 2. Komparacija antioksidativne aktivnosti metanolnih ekstakata bibera

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SAŽETAK

Biber je jedan od najpoznatijih i najraširenijih začina na svijetu. Ova komercijalna biljka pripada obitelji Piperaceae. Ovisno o tretmanu kojem je plod bibera izložen, razlikujemo crni, bijeli i zeleni biber. U ovom istraživanju pripremljeni su metanolni ekstrakti različitih vrsta bibera (*Piper nigrum*) i ružičastog bibera (*Schinus terebinthifolius Raddi*). Maceracijom i ultrazvučnom ekstrakcijom ekstrahirane su bioaktivne komponente iz uzoraka bibera. Redukcijski potencijal ekstrakata testiran je FRAP metodom. Učinkovitost inhibicije slobodnih radikala određena je DPPH metodom. Ekstrakt ružičastog bibera pokazao je najveće antioksidativno djelovanje u *in vitro* uvjetima. Visoko antioksidativno djelovanje zabilježeno je i u ekstraktima zelenog bibera. Općenito, ekstrakti su pokazali visoku moć neutralizacije slobodnih radikala.