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ORIGINAL SCIENTIFIC PAPER

Influence of the Ratio of Water and Organic Solvents on the Antioxidative Activity and Content of Bioactive Components in *Artemisia annua* L. and *Artemisia absinthium* L. Extracts

Utjecaj omjera vode i organskih otapala na antioksidativno djelovanje i sadržaj bioaktivnih komponenti u ekstraktima *Artemisia annua* L. i *Artemisia absinthium* L.

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ABSTRACT

Bitter and sweet wormwoods are traditional plant species in the Asteraceae (Compositae). Their use in traditional medicine has long been known. Numerous preparations of bitter and sweet wormwood (teas, tinctures) are used in the treatment of diseases of the digestive system. The content of bioactive components (polyphenols and flavonoids) and antioxidant activity of *Artemisia absinthium* L. (bitter wormwood) and *Artemisia annua* L. (sweet wormwood) were examined in this paper. A series of extracts were prepared by mixing selected organic solvents (methanol, ethanol and acetone) and water in different volume ratios for both analyzed species. Antioxidant activity was tested using FRAP and DPPH methods. Extracts of sweet wormwood contain more bioactive components and have a higher antioxidant capacity compared to extracts of bitter wormwood. In terms of extraction efficiency, the mixture of acetone and water (20:30 v/v) proved to be the most efficient. Regarding pure organic solvents, the most effective for bioactive components isolation is ethanol, while acetone showed the weakest extraction power.

Keywords: *Wormwood, Polyphenols, Flavonoids, FRAP, DPPH inhibition*

INTRODUCTION – *Uvod*

Wormwoods are a medicinal, aromatic plants from the Artemisia genus, which represents one of the largest and most widespread genera of the Asteraceae (Compositae). According to the literature, this genus includes more than 500 species of aromatic plants (Abad et al., 2012). The two most famous species of this genus are *Artemisia. absinthium* L. (bitter) and *A. annua* L. (sweet) wormwoods. *Artemisia absinthium* is a resistant perennial shrub that is widespread mainly in the temperate zones of Asia, Europe and North America (Beigh and Ganai, 2017). *Arte-*

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misia annua is a fragrant annual weed of Asian origin, but it is naturalized in many other sunny and warm regions (Wan et al., 2016). Wormwoods have a long history of use in folk medicine. Namely, *A. absinthium* has traditionally been used to treat several disorders including hepatocyte enlargement, hepatitis, gastritis, jaundice, wound healing, splenomegaly, dyspepsia, indigestion, flatulence, stomach pain, anaemia, and anorexia (Batiha et al., 2020). In the form of teas or tinctures, it is used to stimulate the appetite and improve digestion, which is a consequence of its bitter taste, which stimulates the secretion of digestive juices. *Artemisia annua* has been used in traditional Chinese medicine to remove toxins from the blood, treat arthritis and fever, prevent the recurrence of malaria and treat jaundice (Qin et al., 2020). Due to the presence of different active substances in the plants, the chemical composition of *A. absinthium* and *A. annua* differs. *Artemisia absinthium* contains many phytochemical compounds, such as lactones, terpenoids, flavonoids, essential oils, organic acids, resins, tannins and phenols, which are responsible for its properties and therapeutic effects (Omer et al., 2007). One of the key compounds the essential oil of *A. absinthium* contains is thujone, a monoterpene ketone. Research has shown that both the α and β forms of thujone in the volatile oil obtained from *A. absinthium* act as anthelmintics (Tariq et al., 2009). The essential oil and aqueous extract of *A. absinthium* have shown analgesic and anti-inflammatory effects as a result of the flavonoids present (Hadi et al., 2014). Also, some studies have shown that isolated chlorogenic acid from *A. absinthium* shows an inhibitory effect on carcinogenesis in the liver, colon and tongue, while another active component, artemisinin, extracted from *A. absinthium* showed a significant antitumor effect against melanoma B16 (Tsuchiya et al., 1996; Goff et al., 2008). Analysis of the chemical composition of *A. annua* found that it contains many phytochemicals, such as monoterpenoids, sesquiterpenoids, flavonoids and coumarins, as well as aliphatic and lipid compounds (Bhakuni et al., 2001). *Artemisia annua* provided a class of highly effective antimalarials due to the presence of the endoperoxide sesquiterpene lactone, artemisinin. This compound is a highly oxygenated sesquiterpene containing a unique 1,2,4-trioxane ring structure, which is responsible for its antimalarial activity (Brown, 2010). Artemisinin-based combination therapies are now considered the best current treatment for uncomplicated *Plasmodium falciparum* malaria (He et al., 2009). The antimalarial efficacy of artemisinin is significantly improved when combined with other compounds such as terpenes, flavonoids, phenolic acids and polysaccharides (Weathers et al., 2011). Artemisinin and its derivatives can also be used in the treatment of various diseases, such as cancers, autoimmune diseases, diabetes, viral infections, parasitosis, and atherosclerosis (Efferth, 2017). Likewise, it was established that artemisinins significantly improve the success rate of chemotherapy (Meng et al., 2021). In addition to artemisinin, *A. annua* also contains many other bioactive components such as monoterpenoids, flavonoids, alkaloids, coumarins, etc. (Septembre-Malaterre et al., 2020). Thanks to its chemical composition, *A. annua* has been the subject of extensive research since its discovery, and it became popular again during the COVID-19 pandemic because it supposedly prevents and helps treat the symptoms of this disease (Irfan et al., 2024). Of the flavonoids present in sweet wormwood, the most significant are artemethin, casticin, chrysosplenetin, chrysosplenol D, cirsilineol and eupatorin, which show synergistic antimalarial effects (Septembre-Malaterre et al., 2020). Phenolic compounds increase the antitumor and antimalarial effects of artemisinin (Ferreira et al., 2010). Scopoline and scopoletin represent the main coumarins found in alcoholic extracts *of A. annua* and contribute to the anti-inflammatory, antioxidant, antipyretic and anti-allergic effects of sweet wormwood (Thabet et al., 2018; Fu et al., 2020).

In this research, the antioxidant activity and the content of bioactive components (polyphenols and flavonoids) of sweet and bitter wormwoods were compared. The influence of the ratio of water and selected organic solvents on the efficiency of extraction of bioactive components in in vitro conditions was also examined.

MATERIALS AND METHODS – *Materijal i metode*

Plant material, chemicals and instruments

Dried aerial parts of bitter and sweet wormwood were purchased in a local market in Tuzla, Bosnia and Herzegovina. The sample was determined in the pharmacognosy laboratory of the Faculty of Pharmacy, University of Tuzla. The plant was ground into powder using an electric mill. Aqueous solutions needed for the analysis were prepared using demineralized water. All reagents were p.a. purity and were used without further purification. Spectrophotometric measurements were performed on a Perkin Elmer Lambda 25 spectrophotometer, in the wavelength range of 510-765 nm.

Preparation of extracts

Extracts of sweet and bitter wormwood were prepared by mixing 0.5 grams of chopped plant material with 50 mL of solvent or solvent mixture. The plant material was mixed on a vibromix for 60 minutes, then the mixture was filtered and the collected extract was analyzed immediately. All extracts were clear after filtration.

Determination of total phenolic content (TPC)

Total phenolic compounds present in the extracts were quantified spectrophotometrically using the Folin-Ciocalteu test following the protocol of Singleton et al. (1999), with some modifications. Namely, 200 µL of extracts was mixed with 2540 µL of 10% Folin-Ciocalteu reagent. After 5 minutes 420 µL of 10% sodium carbonate was added. The absorbance of the resulting blue-coloured solution was measured at 765 nm after incubation at room temperature for 1 hour. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry plant material.

Determination of total flavonoid content (TFC)

The total flavonoid content in the extracts was determined by the previously described method of Olajire and Azeez (2011), with some modifications. Namely, 1 mL of extract solution was mixed with 0.3 mL of 5% sodium nitrite, and 0.3 mL of 10% aluminium chloride was added after 5 minutes. After 6 minutes of incubation at room temperature, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 mL with distilled water. The absorbance of the sample was measured against the blank at 510 nm using a spectrophotometer. The results were derived from the calibration curve of quercetin and expressed in quercetin equivalents (QE) per gram of dry plant material.

Ferric-reducing antioxidant power (FRAP) Assay

The reducing powers of the extracts that reflected their antioxidant activity were determined following the protocol of Benzie and Strain (1999). Per it, 3 mL of prepared FRAP reagent is mixed with 100 µL of extracts. Absorbances at 593 nm are recorded after a 30-minute incubation at 37 °C. The FRAP value was calculated from the calibration curve of iron(II) sulfate heptahydrate and expressed in mol per gram of dry plant material.

DPPH radical scavenging activity

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to the earlier described method of Horozić et al. (2019). The percentage of DPPH radical inhibition was tested by mixing 2 mL of 0.5 mg/mL extract solution with 0.5 mL of 0.5 mM DPPH radical solution. The samples were left to incubate for 30 minutes in a darkened room at room temperature. As a control sample 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used. The radical scavenging

Table 1. Results of the content of bioactive components and antioxidant activity of *Artemisia annua* L. extracts

Tabela 1. Rezultati sadržaja bioaktivnih komponenti i antioksidativne aktivnosti ekstrakata Artemisia annua L.

Sample	Extraction solvent	Solvent ratio (v/v)	TPC [mg GAE/g DW]	TFC [mg QE/g DW]	FRAP value [µmol/g DW]	DPPH inhibition [%]
A-1	EtOH	-	11.05	0.013	115.7	71.25
A-2	EtOH:Water	40:10	16.27	0.028	289.7	80.50
A-3	EtOH:Water	30:20	20.24	0.037	353.0	85.47
A-4	EtOH:Water	20:30	23.03	0.039	375.1	88.94
A-5	EtOH:Water	10:40	17.05	0.034	321.5	81.80
A-6	MeOH	-	10.12	0.017	112.5	56.25
A-7	MeOH:Water	40:10	15.77	0.029	283.5	61.67
A-8	MeOH:Water	30:20	19.31	0.033	339.1	68.02
A-9	MeOH:Water	20:30	20.41	0.036	342.5	74.20
A-10	MeOH:Water	10:40	16.86	0.031	315.8	64.96
A-11	Ace	-	1.86	0.005	67.3	5.86
A-12	Ace:Water	40:10	14.62	0.029	319.0	93.85
A-13	Ace:Water	30:20	19.36	0.041	357.0	95.77
A-14	Ace:Water	20:30	24.08	0.044	392.3	97.05
A-15	Ace:Water	10:40	19.15	0.038	331.1	94.57
A-16	Water	-	14.74	0.035	120.4	84.46

*TPC - Total phenolic content; TFC - Total flavonoid content, FRAP - Ferric-reducing antioxidant power

effect (%) or percentual inhibition of DPPH radical was calculated according to the equation:

[(Ac - As) / Ac] x 100

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

RESULTS AND DISCUSSION – *Rezultati i diskusija*

Tables 1 and 2 show the results of the analysis of the content of polyphenols and flavonoids, as well as the antioxidant capacity of extracts of sweet and bitter wormwoods. It is clearly noticeable that bitter wormwood contains fewer bioactive components and reflects lower antioxidant activity compared to extracts of sweet wormwood. The results of the content of total phenolic compounds and flavonoids in the extracts and the antioxidant activity of the extracts indicate their significant correlation.

In the extraction of bioactive components from both plant species, mixtures of organic solvent and water, in a volume ratio of 20:30 v/v were proved to be the most effective. The concentrations of the determined bioactive components were increased by the water content in all used solvents from 40:10 v/v to 20:30 v/v, while the concentration of the investigated components decreased in extracts with an increased water content to 10:40 v/v. Acetone mixtures generally proved to be more efficient in the extraction of bioactive components compared to ethanol and methanol mixtures. In terms of pure solvents, the most efficient extraction was achieved with water, followed by ethanol, and methanol, and the weakest extraction effect was recorded for acetone extracts.

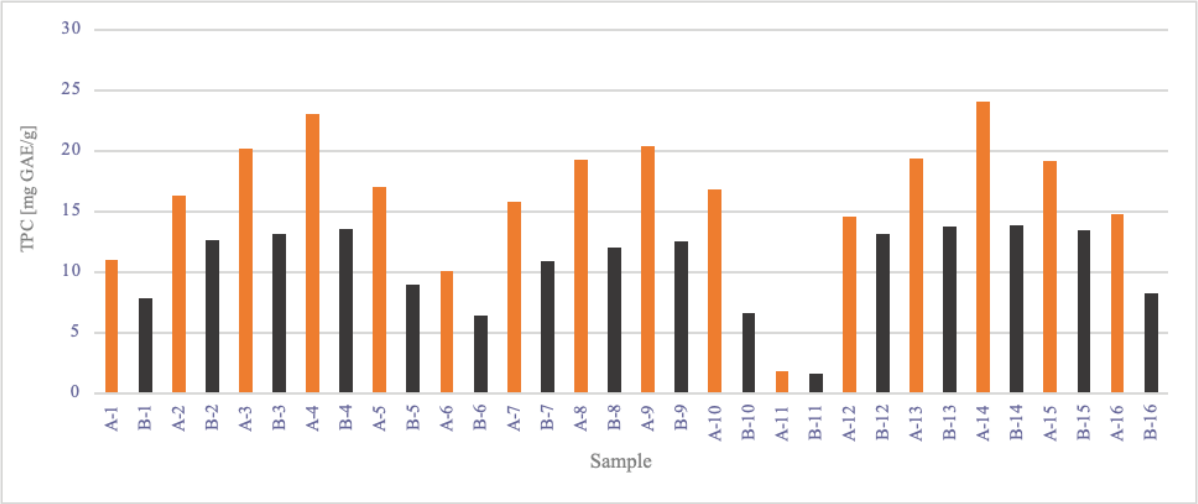
A minimally invasive extraction technique was used, which preserved a significant proportion of polyphenols, which explains the relatively high values of the proportion of the mentioned components in the extracts. Extraction efficiency is enhanced by the small particle size, which improves solvent penetration and solute diffusion. In general, the finer the particle size, the better the extraction results (Begić et al., 2020). During the extraction of dry plant material, a better effect is achieved with a higher proportion of the aqueous phase in the organic phase (Bimakr et al., 2011), which explains the higher extraction capacity of mixtures of organic solvents and water in this research. Altiok et al. (2008) confirmed the importance of the presence of water in the organic solvent, which increases the diffusi-

Table 2. Results of content of bioactive components and antioxidant activity of *Artemisia absinthium* L. extracts

Tabela 2. Rezultati sadržaja bioaktivnih komponenti i antioksidativne aktivnosti ekstrakata Artemisia absinthium L.

Sample	Extraction solvent	Solvent ratio (v/v)	TPC [mg GAE/g DW]	TFC [mg QE/g DW]	FRAP [µmol/g DW]	DPPH inhibition [%]
B-1	EtOH	-	7.83	0.014	94.55	47.92
B-2	EtOH:Water	40:10	12.60	0.016	135.44	78.43
B-3	EtOH:Water	30:20	13.12	0.017	164.54	80.34
B-4	EtOH:Water	20:30	13.55	0.018	224.12	84.92
B-5	EtOH:Water	10:40	8.96	0.016	141.50	59.14
B-6	MeOH	-	6.45	0.014	84.24	45.15
B-7	MeOH:Water	40:10	10.91	0.016	122.8	60.01
B-8	MeOH:Water	30:20	11.98	0.017	191.2	66.21
B-9	MeOH:Water	20:30	12.58	0.017	195.4	70.25
B-10	MeOH:Water	10:40	6.57	0.016	131.1	56.95
B-11	Ace	-	1.58	0.005	58.28	4.99
B-12	Ace:Water	40:10	13.15	0.018	206.84	69.23
B-13	Ace:Water	30:20	13.75	0.019	241.44	88.84
B-14	Ace:Water	20:30	13.90	0.020	256.24	92.11
B-15	Ace:Water	10:40	13.50	0.019	221.22	68.75
B-16	Water	-	8.27	0.014	95.84	48.82

*TPC - Total phenolic content; TFC - Total flavonoid content, FRAP - Ferric-reducing antioxidant power



Graph 1. Comparison of polyphenol content in *Artemisia annua* and *A. absinthium* extracts

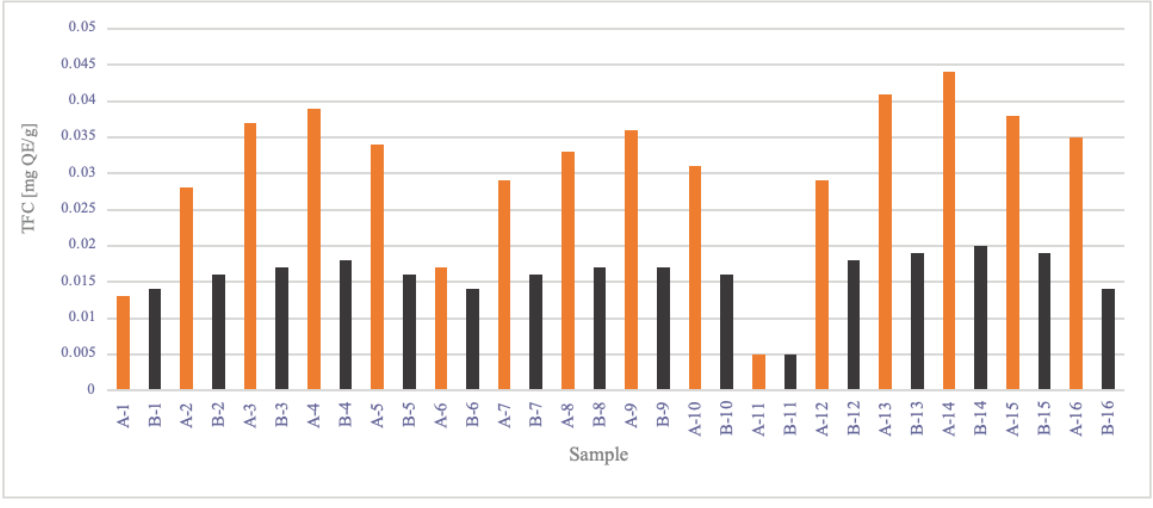
Grafikon 1. Komparacija sadržaja polifenola u ekstraktima Artemisia annua i A. absinthium

on process and thus facilitates the extraction of phenolic compounds from plant tissue.

Extraction of flavonoids of the studied wormwoods species showed a strong correlation with the polarity of the solvents used. These results agree with Spingo et al. (2007), who suggested that polar solvents are the best medium for flavonoid extraction, which may be due to the increase in polarity of flavonoids after conjugation via glycosides with hydroxyl groups, which increases their solubility in polar solvents (Mohsen and Ammar, 2009). The trend of phenol extraction among different solvents was similar to that of flavonoids, i.e. phenols were more efficiently extracted in polar solvents, specifically in water, ethanol and methanol compared to acetone, which proved to be the weakest medium for their extraction.

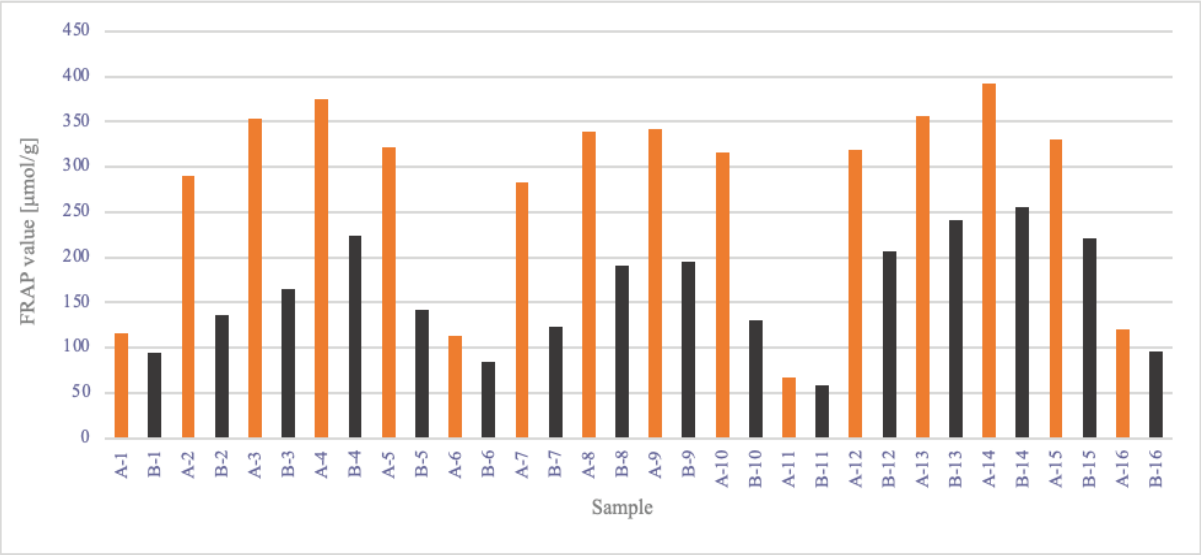
Comparative diagrams of the content of bioactive components and antioxidant capacity in *in vitro* conditions for extracts of sweet and bitter wormwoods are shown in Graphs 1 to 4.

The solvents and polarity used in the extraction process can influence the amount of extracted bioactive compounds, as well as the value of antioxidant activity (Budiana et al., 2017). The results obtained on the basis of FRAP and DPPH analysis of the extracts of the analysed



Graph 2. Comparison of flavonoid content in *Artemisia annua* and *A. absinthium* extracts

Grafikon 2. Komparacija sadržaja flavonoida u ekstraktima Artemisia annua i A. absinthium

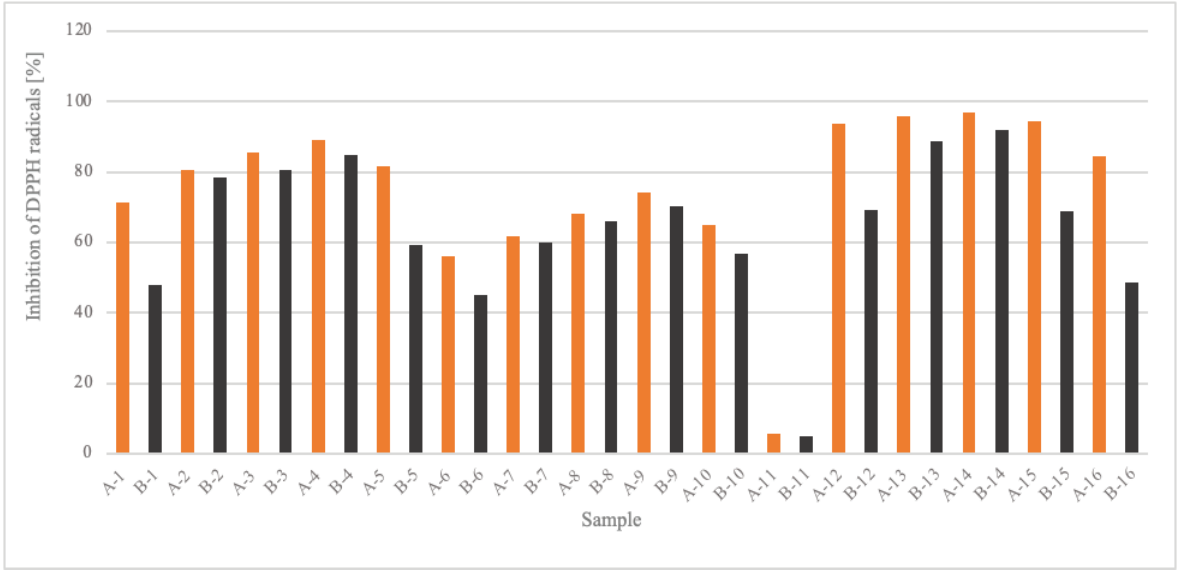


Graph 3. Comparison of the reduction potential of *Artemisia annua* and *A. absinthium* extracts

Grafikon 3. Komparacija redukcijskog potencijala ekstrakata Artemisia annua i A. absinthium

wormwood species obtained with pure solvents indicate that the best antioxidant activity was achieved with water and the weakest with acetone. The antioxidant activity of ethanolic extracts was higher than methanolic extracts. In general, with an increase in the water content, the obtained ethanolic, methanolic and acetone extracts of analyzed *Artemisia* species show an increase in antioxidant activity, respectively, except for the 10:40 v/v extract mixtures, which showed a moderate decrease.

The antioxidant activity of analyzed *Artemisia* species was also examined in a study conducted by Sembiring et al. (2022). The ethanol and methanol solvents in different concentrations and water as a control were used to prepare the extracts. The type and concentration of the solvent significantly influenced the yield and antioxidant activity of the *Artemisia* extract. The use of ethanol as a solvent resulted in a higher extract yield and antioxidant activity than methanol. The antioxidant activity of the ethanolic extract of *Artemisia* was stronger than the methanolic extract (Sembiring et al., 2022).



Graph 4. Comparison of DPPH radical inhibition by *Artemisia annua* and *A. absinthium* extracts

Grafikon 4. Komparacija inhibicije DPPH radikala ekstrakata Artemisia annua i A. absinthium

CONCLUSIONS – *Zaključak*

This research can help in the preparation of extracts of other plant species because it gives a more detailed insight into the efficiency of mixtures of organic solvents and water, as well as the ideal proportions of water and organic solvent that extract the highest content of polyphenols and flavonoids. It is important to emphasize that the mentioned mixtures cannot be effective for all plant species and samples, which is why there is a great interest in this type of research.

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SAŽETAK

Gorki (*Artemisia absinthium* L.) i slatki pelin (*A. annua L.*) tradicionalne su biljne vrste u porodici Asteraceae (Compositae). Njihova upotreba u tradicionalnoj medicini odavno je poznata jer se čajevi i tinkture spravljeni od njih koriste u liječenju bolesti probavnog sistema. U radu je ispitan sadržaj bioaktivnih komponenti (polifenola i flavonoida) i antioksidativno djelovanje (FRAP i DPPH metodom) navedenih vrsta. Za analizirane vrste pripremljena je serija ekstrakata s odabranim rastvaračima (voda, metanol, etanol i aceton) te smjesama navedenih organskih rastvarača s vodom u različitim volumnim omjerima. Rezultati istraživanja su pokazali da ekstrakti slatkog pelina imaju više bioaktivnih komponenti, a time i izraženije antioksidativno djelovanje u odnosu na ekstrakte gorkog pelina. U smislu učinkovitosti ekstrakcije, najučinkovitijom se pokazala mješavina acetona i vode (20:30 v/v). Što se tiče čistih organskih otapala, najučinkovitija za izolaciju bioaktivnih komponenti je voda, dok je aceton pokazao najslabiju ekstrakcijsku moć.

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