Glasnik hemičara i tehnologa Bosne i Hercegovine

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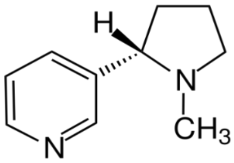
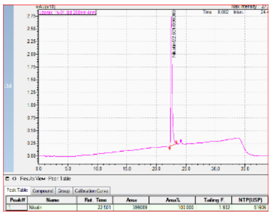
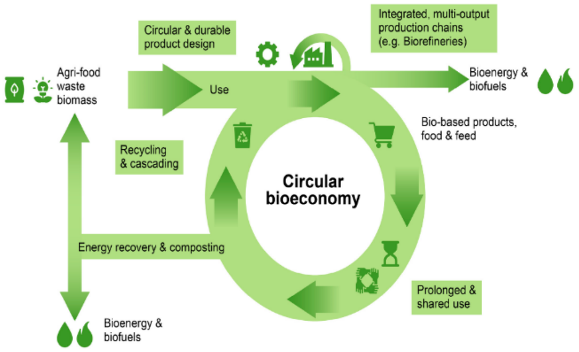
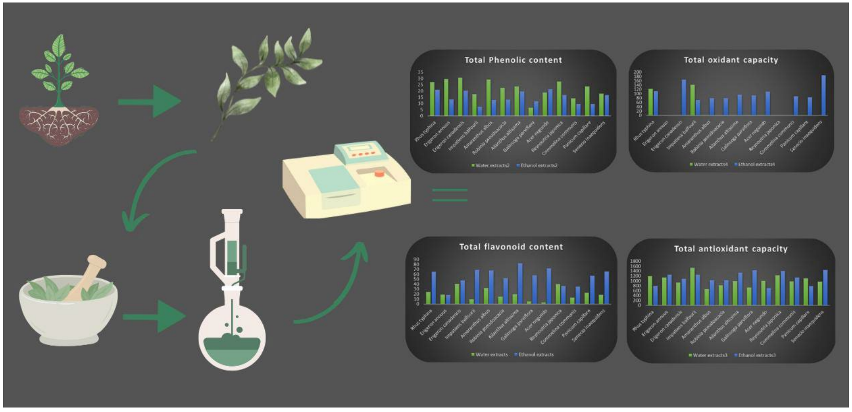
ORIGINAL SCIENTIFIC ARTICLES







PROCEEDINGS



Raspberry	TPs ± SD (mg GAE/100 g)	TPs ± SD (mg GAE/100 mL)
„Tulameen“	**1608.77 ± 72.71**	1003.30 ± 156.98
„Willamette“	1604.81 ± 33.01	998.40 ± 72.07
„Fertodi“	1525.01 ± 65.65	423.27 ± 38.36
W-sc*	1504.09 ± 26.97	**1417.65 ± 132.76**
„Meeker“	1457.06 ± 56.27	1006.45 ± 166.02
„Polka“	1443.82 ± 36.50	154.66 ± 23.10

Editorial

Dead Bees - A Warning That We Must Not Ignore

According to data from the American National Institute for Food and Agriculture, the death of bees has negative consequences for the entire society, considering that about 35% of the world's food depends on pollinators. Bees are certainly one of the most important pollinators. In 2025, a significant number of dead bees was recorded around the world, including in Bosnia and Herzegovina. In the United States, the percentage of dead bees was 50-70%. In Serbia, the loss of bee colonies varied from region to region, but the greatest loss was in Vojvodina and western Serbia. Losses of bee colonies from 35% to 100% have been recorded in Serbia. In Bosnia and Herzegovina, that percentage is between 30% and 50%, although there are also losses in the percentage of 90%.

The death of about 10% of bees after the winter period is something that usually happens because there comes a period when the winter bees need to raise the first generation of spring bees. If the bee colonies are weakened and do not have enough strength to carry out that shift, the death of the old bees occurs before they pass the role to the new generations, but this certainly does not happen in such a large percentage as it did in 2025. Therefore, the question arises as to what is the reason for such a large and massive death of bees. Taking into account the fact that the beekeepers state that there are no dead bees in front of the hives or even inside the hives, but the hives are empty and inside the hives there are few reserves of honey and pollen and very little honey. What is certain is that the death of the bees did not occur only because of one factor, but that several of them led to this outcome. Potential causes of bee death are: varroa, viruses, pesticides, insecticides, climate change leading to large dry periods resulting in poor bee nutrition, the appearance of invasive hornet species, habitat degradation, and stress. Sublethal doses of immunosuppressive pesticides favor the spread of bee diseases, and pesticides and their interactions contribute to the loss of bee colonies caused by stress. Neurotoxic insecticide molecules affect the cognitive abilities of bees, reducing their performance and ultimately affecting the sustainability of bee colonies. Immunosuppression of natural defenses by neonicotinoid and phenyl-pyrazole (fipronil) insecticides opens the way for parasitic infections and viral diseases, encouraging their spread among individuals and among bee colonies at higher rates than in conditions without exposure to such insecticides. Synergistic interactions between the parasitic Varroa mite and viral pathogens severely reduce host immunity and contribute to the deterioration of bee health. The long dry period that is a consequence of climate change also has a negative effect on bees because it leads to impoverished bee nutrition, and bees that do not have enough food are more susceptible to virus infections. Pollen is very important for the nutrition of bees because it is rich in proteins, amino acids, vitamins, and minerals that are necessary for the proper growth and development of bees, and in case of its deficiency, bees' immunity weakens and bees can die.

Although many factors are contributing to the global decline of honeybee populations, the most dangerous stressor is the Varroa mite. The Varroa mite is a parasite that attacks adult honeybees and their brood and feeds on hemolymph, which weakens their immune system. Varroa reproduces quickly and can overwhelm a honey bee colony in about six months. The presence of varroa in the hive leads to:

- reduction of the total population of the colony, which affects the possibilities of nutrition and pollination,

- decrease in honey production due to reduced nutrition and damaged health of worker bees,

- weakening of colonies and increasing their sensitivity to additional stressors from the environment.

The mass death of bees recorded in 2025 should be a serious alarm for the population, the environment, agriculture, and food security. Of particular concern is the fact that bee colonies are disappearing without obvious signs of death, which may indicate multiple stressors acting simultaneously, from parasites and viruses to the use of pesticides to climate change and habitat degradation. This is a complex problem that requires coordinated action to protect bees as one of the key pollinators and thus ensure the stability of the ecosystem and agricultural production. Some of the recommendations that could certainly contribute to the protection of bees are certainly limiting the use of pesticides and insecticides, controlling their use/banning them, or using biologically acceptable methods of treating plants during the flowering phase. Improving the habitat and nutrition of bees by planting honey plants would create more comfortable conditions for bees. Control and suppression of varroa and other parasites through regular monitoring and treatment of bee colonies using effective and bee-harmless means. Monitoring of climate changes and timely notifications of extreme weather conditions, education and training of beekeepers, well as cooperation with the scientific sector and researchers.

Editors

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01-05

Evaluation of Nicotine Transdermal Patches Available on the Domestic Market

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Abstract: The use of transdermal patches with nicotine is a better way to quit smoking because it ensures a similar concentration of nicotine in the blood as when smoking and reduces the morning desire for a cigarette. The aim of this work was to analyze the behavior of the matrix with the active substance under conditions that are close to skin conditions. A total of 3 samples were collected from local market. The analysis was performed after 0.5, 1, 4, 10, 16 and 24 hours for each sample. The concentration of released nicotine for samples at 50 RPM ranged in the following range: sample 1 43.87 %-115.23 %; sample 2 40.56 %-114.70 %; sample 3 43.53 %-117.13 %. The concentration of released nicotine for the samples at 100 RPM ranged in the following range: sample 1 45.14 %-120.82 %; sample 2 49.05 %-120.79 %; sample 3 44.73 %-118.51 %. It was determined that most of the nicotine is released already after 4 hours. The samples also show very similar concentration results after 10 hours, and for all three samples the result was 0.096 mg/ml.

INTRODUCTION

Nicotine is the main psychoactive compound in tobacco and has driven its widespread use since ancient times. It is a tertiary amine composed of pyridine and pyrrolidine rings (Figure 1). Predominantly found in its (S)-nicotine form, it can constitute up to 3% of dried Nicotiana tabacum leaves.In some tobacco species, such as Nicotiana rustica, it can be present in much higher concentrations, around 14% (Sansone et al., 2023; Benowitz et al., 2009).

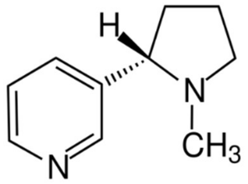

Figure 1: Chemical structure of nicotine

Nicotinic acetylcholine receptors are members of a superfamily of ligand-gated pentameric ion channels, and are widely distributed in the living world, from plants to mammals. These receptors can have different roles that depend on their localization in the tissue. In neural tissues, they are involved in cognition, addiction and cell growth, while present in other tissues, they contribute to various functions, such as inflammation and immunity (Garduño-Sánchez et al., 2023). The biological effects of nicotine are diverse and include negative effects on the cardiovascular system and addiction, as well as positive effects, such as improving cognitive functions in some conditions (Hall et al., 2014; Wang et al., 2023).

Once tobacco smoke reaches the alveoli, nicotine is rapidly absorbed, and blood concentrations rise rapidly during smoking. After inhalation, large amounts reach the brain within about 10 to 20 seconds, crossing the blood-brain barrier.Nicotine is also well absorbed through the skin. It is extensively metabolized in the liver, and six primary metabolites have been identified. It is excreted by glomerular filtration and tubular secretion, thus largely via the kidneys (Benowitz et al., 2009).

Nicotine can be used in experimental pharmacology, as a pesticide, and to alleviate withdrawal symptoms, or as an aid to smoking cessation. Various formulations of nicotine replacement therapy, such as nicotine gum, transdermal patch, nasal spray, inhaler, sublingual tablets, and lozenges, are commercially available and are intended for the indication of smoking cessation (Choi et al., 2003).

Transdermal patches represent a special pharmaceutical form of a drug and can be used in various indications. By using this form, it is possible to avoid numerous problems associated with, for example, oral administration of an active principle, such as: first-pass metabolism, degradation by digestive enzymes, degradation of the drug in the acidic gastric medium, irritation of the gastrointestinal mucosa, etc. It also enables use in patients who cannot receive the drug in another way or when a specific condition requires gradual release of the drug over a longer period of time. Currently, different transdermal patches are available on the market for use in the treatment of smoking cessation, pain relief, osteoporosis, contraceptive patches, angina pectoris and cardiac disorders. However, formulations that would be more suitable for the delivery of some more challenging drugs are in various stages of development. When formulating transdermal patches, special attention should be paid to the physicochemical properties of the active and inactive components, as well as the possibility of application over a longer period of time (Al Hanibali et al., 2019).

The use of nicotine patches compared to chewing gum has certain advantages, as it primarily provides therapeutic levels of nicotine from the first day of treatment with minimal effort. Patches are commercially available in three doses (sizes) and most smokers start with the highest dose for the first 4 to 8 weeks, followed by a systemic withdrawal period of 2 to 8 weeks. During this period, progressively smaller doses are used. Two types of nicotine patches are available, reservoir and matrix passive diffusion, where one type is worn only when awake, during the day, with the highest dose of 15 mg of nicotine during 16 hours, and the other can be worn both during the day and overnight, and delivers 21 mg of nicotine during 24 hours (Jarvis and Sutherland, 1998).

The aim of this work was to study the scientific literature and provide the latest information in the field transdermal applications of nicotine. The main aims included the following: determining the release of contents from the patch sample using the HPLC method, measure the percentage and concentration of released nicotine by sampling points, and compare and discuss the obtained results.

EXPERIMENTAL

Chemicals

The following chemicals and reagents were used for purposes of analysis: ammonium acetate (Carlo Erba), ammonium hydroxide (Sigma Aldrich), potassium hydroxide (Merck), acetonitrile, HPLC purity (Fischer chemical).

Apparatus and methods

For the dissolution of transdermal patches in the European Pharmacopoeia (Ph. Eur. XI), three devices are listed: apparatus with a blade above the disc, apparatus with a spatula above the cell and rotating cylinder apparatus. Which apparatus will be used depends on the composition, type and dimensions of the patch (Council of Europe, 2023). In this work, an apparatus with a spatula above the disk was used (Figure 2.).

The principle of the disk is to hold the patch at the bottom of the glass of the dissolution apparatus, whereby the space between the bottom of the glass and the disk is minimal. The disk keeps the patch flat and facing the scapula. The distance between the blade and the disk is 25±2 mm. The medium temperature is 32±0.5 °C (skin temperature). The glasses are covered to reduce evaporation of the medium.

The analysis was performed on an Erweka DT 826 dissolution apparatus. As a dissolving medium potassium dihydrogen phosphate buffer, pH = 5.0 was used. The phosphate buffer is made by dissolving 2.72 g of KH_2PO_4 in 1000 ml of water, and adjusting the pH with a 1 M solution of potassium hydroxide (KOH). Blade rotation speed was 50 and 100 revolutions per minute. The total duration of the dissolution was 24 hours, with sampling at several points starting from the 30th minute and after 1, 4, 10, 16 and 24 hours. Medium volume was 500 ml and sampling volume was 10 ml with medium change.

Six glasses of the dissolution apparatus were filled with a specified volume of medium. The apparatus parameters were then set, and the system was allowed to stabilize at the designated values. Once stabilized, six discs containing properly placed samples were immersed in the glasses, initiating the analysis.

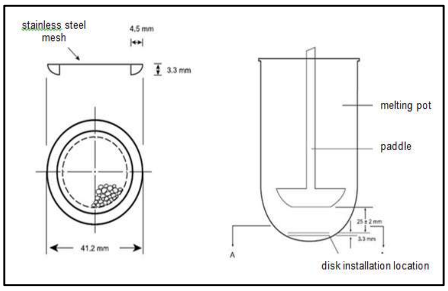

Figure 2: Apparatus with a spatula above the disk

To set the chromatographic parameters, a modified HPLC method was used for rapid and simultaneous determination of nicotine (Jablonski et al., 2006; Vlasceanu et al., 2016).

Table 1: HPLC analysis parameters

MP A	Acetate buffer (pH = 10)	Oven temperature	40 °C
MP B	Acetonitrile	Autosampler temperature	20-25 °C
Flow rate of MP	1.8 ml/min	Sample injection volume	25µl
Detection (UV)	260 nm	Analysis time	40 min

Gradient program of the mobile phase for HPLC separation

Time (min)	MP A (vol %)	MP B (vol%)
0	100	0
30	65	35
32	100	0
40	100	0

Samples

The Patches containing nicotine as an active substance were used for the analysis and were randomly marked as Sample 1-3. The following commercially available preparations were tested: Nicorette 15 mg/16 h, Nicorette 25 mg/16 h and NicoretteSkin 25 mg/16h (Figure 3.).

Figure 3: Test samples (Nicorette 15mg/16h, Nicorette 25mg/16h and NicoretteSkin 25mg/16h)

RESULTS AND DISSCUSION

Release profiles of samples at 50 RPM

For the first sample analysis, a medium volume of 500 ml was selected, and the speed of rotation of the paddle per minute was set to 50 RPM. After the medium was prepared and the apparatus reached the set parameters, the analysis of six samples was started. The autosampler is set to sample after 30 minutes, then after 1, 4, 10, 16 and after 24 hours. After dissolution, the analysis was continued on the HPLC apparatus. The chromatograms of all samples are shown in Figure 4. Results are shown in Table 2.

Table 2: Released nicotine (%) in different time intervals at 50 RPM

#	Time (h)	Sample 1 (% of nicotine)	Sample 2 (% of nicotine)	Sample 3 (% of nicotine)
1	0.5	43.86	40.56	43.53
2	1	61.06	60.89	60.72
3	4	99.43	97.74	100.06
4	10	111.20	109.69	111.76
5	16	113.26	112.14	114.75
6	24	115.23	114.70	117.13

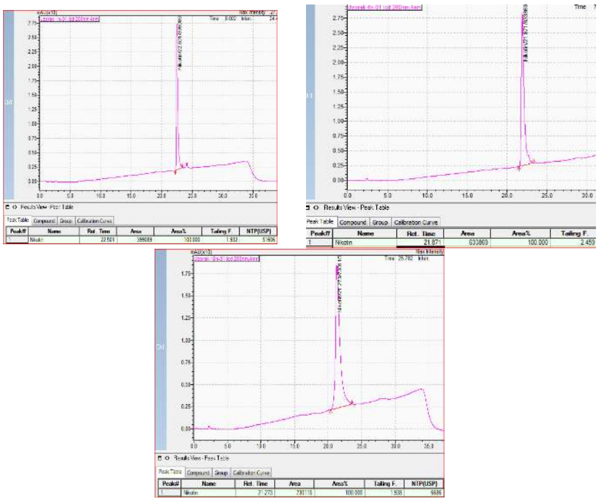

Figure 4: Chromatograms of samples

Release profiles of samples at 100 RPM

For the second sample analysis, a medium volume of 500 ml was selected, and the speed of rotation of the paddle per minute was set to 100 RPM. After the medium was prepared and the apparatus reached the set parameters, the analysis of six samples was started. The autosampler is set to sample after 30 minutes, then after 1, 4, 10, 16 and after 24 hours. After dissolution, the analysis was continued on the HPLC apparatus.

Results are shown in Table 3.

Table 3: Released nicotine (%) in different time intervals at 100 RPM

#	Time (h)	Sample 1 (% of nicotine)	Sample 2 (% of nicotine)	Sample 3 (% of nicotine)
1	0.5	45.15	46.05	44.73
2	1	62.95	65.92	62.27
3	4	103.85	104.35	102.50
4	10	116.33	116.05	113.16
5	16	118.70	118.08	115.91
6	24	120.82	120.79	118.31

Comparison of results at 50 RPM and 100 RPM

Table 4. and Table 5. show RSD values for all samples at 50 RPM and 100 RPM.

Table 4: RSD (%) values in different time intervals at 50 RPM

#	Time (h)	Sample 1 (% RSD)	Sample 2 (% RSD)	Sample 3 (% RSD)
1	0.5	0.98	3.95	1.68
2	1	1.07	1.48	1.64
3	4	1.14	1.36	1.29
4	10	1.19	1.52	0.84
5	16	1.18	1.64	1.35
1	0.5	0.98	3.95	1.68

Table 5: RSD (%) values in different time intervals at 100 RPM

#	Time (h)	Sample 1 (% RSD)	Sample 2 (% RSD)	Sample 3 (% RSD)
1	0.5	2.16	2.45	3.62
2	1	1.37	2.21	3.64
3	4	1.48	2.12	2.84
4	10	1.46	2.15	2.42
5	16	1.61	234	2.70
6	0.5	2.16	2.45	3.62

The RSD values of the samples show a rather uneven release of nicotine. If we follow the % RSD values, we can see that Sample 2 at RMP 50 has a large deviation, which is not the case for the other two Samples. High RSD values are not related to a specific sample, but are randomly scattered, which indicates possible differences in the patch formulations.

In Table 6. is shown average concentration (mg/ml) of released nicotine for all samples and time intervals at 50 RPM and 100 RPM. Figure 5. and Figure 6. show graphically the nicotine release profiles of all samples in the given time intervals.

Table 6: Average concentration (mg/ml) of released nicotine for all samples and time intervals at 50 RPM and 100 RPM

50 RPM				
#	Time (h)	Sample 1 (mg/ml)	Sample 2 (mg/ml)	Sample 3 (mg/ml)
1	0.5	0.038	0.035	0.038
2	1	0.052	0.052	0.052
3	4	0.083	0.082	0.084
4	10	0.092	0.091	0.092
5	16	0.092	0.091	0.093
1	0.5	0.092	0.091	0.093
100 RPM				
#	Time (h)	Sample 1 (mg/ml)	Sample 2 (mg/ml)	Sample 3 (mg/ml)
1	0.5	0.039	0.042	0.039
2	1	0.054	0.056	0.053
3	4	0.087	0.088	0.086
4	10	0.096	0.096	0.094
5	16	0.096	0.096	0.094
1	0.5	0.096	0.096	0.094

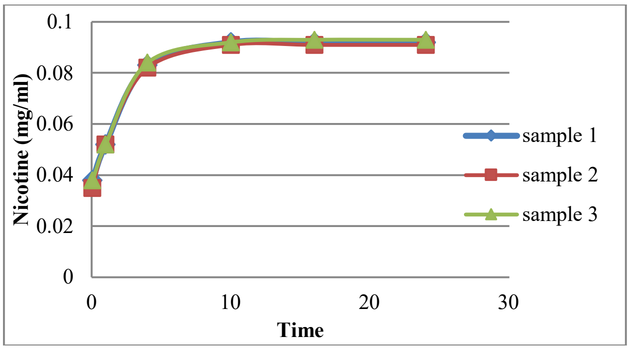

Figure 5: Graphical representation of released nicotine (mg/ml) in different time intervals at 50 RPM

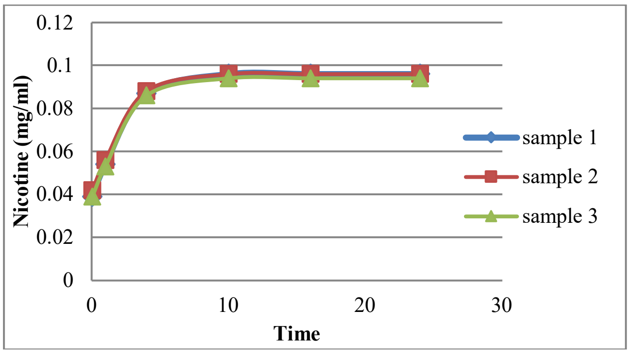

Figure 6: Graphical representation of released nicotine (mg/ml) in different time intervals at 50 RPM

CONCLUSION

Transdermal administration offers precise control over drug levels in the blood, allows for less frequent dosing, bypasses first-pass metabolism in the liver and gastrointestinal inactivation, enables easy treatment discontinuation, and provides a non-invasive method of drug delivery. Because of the advantages that transdermal application offers more than other methods of drug administration, intensive work is being done on its development. Disadvantages of transdermal patches are high production costs, which ultimately lead to a higher product price and aesthetic acceptability.

The aim of this work was to analyze the behavior of the matrix with the active substance under conditions that are close to skin conditions, and then to find a suitable method for HPLC determination of the content of the active substance in the samples. A total of 3 samples were collected from local market. The analysis was performed after 0.5, 1, 4, 10, 16 and 24 hours for each sample, and at revolutions of 50 RPM and 100 RPM. The concentration of released nicotine for samples at 50 RPM ranged in the following range (for the indicated time intervals): sample 1 43.87%-115.23%; sample 2 40.56%-114.70%; sample 3 43.53 %-117.13 %. The concentration of released nicotine for the samples at 100 RPM ranged in the following range (for the indicated time intervals): sample 1 45.14%-120.82%; sample 2 49.05%-120.79%; sample 3 44.73 %-118.51 %. It was determined that most of the nicotine is released already after 4 hours. The samples also show very similar concentration results after 10 hours, and for all three samples the result was 0.096 mg/ml.

A slightly different HPLC method was adopted and used in this study for a fast and simple screening of nicotine from transdermal patches. These tests have shown that on the basis of the used pharmacopoeia test „Paddle over Disc“ it is possible to successfully compare different formulations from the market and evaluate their quality.

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Summary/Sažetak

Upotreba transdermalnih flastera s nikotinom je bolji način za prestanak pušenja, jer osigurava sličnu koncentraciju nikotina u krvi kao kod pušenja i smanjuje jutarnju želju za cigaretom. Cilj ovog rada bio je da se ispita ponašanje matriksa s aktivnom supstancom u uslovima koji su bliski uslovima kože. Ukupno su prikupljena 3 uzorka s lokalnog tržišta. Analiza je obavljena nakon 0,5, 1, 4, 10, 16 i 24 sata za svaki uzorak. Koncentracija oslobođenog nikotina za uzorke na 50 obrtaja/min kretala se u sljedećem rasponu: uzorak 1 43,87 %-115,23 %; uzorak 2 40,56 %-114,70 %; uzorak 3 43,53 %-117,13 %. Koncentracija oslobođenog nikotina za uzorke pri 100 obrtaja/min kretala se u sljedećem rasponu: uzorak 1 45,14 %-120,82 %; uzorak 2 49,05 %-120,79 %; uzorak 3 44,73 %-118,51 %. Utvrđeno je da se većina nikotina oslobađa već nakon 4 sata. Uzorci također pokazuju vrlo slične rezultate po pitanju koncentracije nikotina nakon 10 sati, a za sva tri uzorka rezultat je bio 0,096 mg/ml.

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Bioeconomy and the Modern Challenge of Sustainable Production and Consumption of Biomass and Biofuels

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Abstract: The bioeconomy encompasses the whole economy's reliance on biological and renewable resources, appears as a response to the global challenges confronting the present civilization. This paper explains the genesis of this concept, focusing particularly on its fundamental components, i.e. the sustainable utilization of biomass and the production of biofuels. It offers an overview of global policies and strategies in this domain, alongside the potential applications of biomass. A large number of studies highlight the great potential of biomass, surpassing the current human needs. However, its application has to be examined through three aspects: supply, demand and sustainability. The increase in biofuel production also imposes challenges such as the competitive use of land for biomass and food production, which is a particular problem, and in this regard, various technologies have been developed that use inedible biomass in the production of biofuels and valuable chemicals. Recognizing the importance of the bioeconomy, as an inevitable element in achieving sustainable development, the European Union adopted a strategy and action plan for the bioeconomy in 2012. Also, many countries that are important players in the global economy, such as the United States, Germany, Canada, Japan, etc. have adopted their national strategies that promote the bioeconomy. It is clear that there is a global interest in research and investment in supply chains for biomass and biofuels, which gives an optimistic picture of the future use of biomass as the basis of a future global bioeconomy.

INTRODUCTION

Today we live in a world of limited resources, with numerous global challenges such as climate change, land and ecosystems degradation, with a steady increase of population. All of this forces us to look for new ways of production and consumption that respect the ecological boundaries of our planet, while achieving sustainability. Facing such challenges gives a strong incentive for the modernization of the industry, a new approach to research and development of processes, and a general shift and orientation towards renewable, biological raw materials. This new approach to production and consumption is often called the bioeconomy.

The bioeconomy encompasses all sectors and systems that rely on biological resources, such as plants, animals, microorganisms and waste of organic origin, and their functioning and principles (EC, 2018a). It can be said that these are economy sectors that derive most of their market value from biological products and/or processes derived from natural materials, as opposed to products and processes based on nonrenewable resources and purely chemical processes (UNIDO & SEI, 2005). Bioeconomy services accounted for between 5.0–8.6% of EU gross domestic product and 10.2–16.9% the EU labor force (Ronzon et al., 2022). From this point of view, the bioeconomy itself is not new, as various economies, before the industrial revolution and the massive use of fossil resources, oil and coal, were mainly based on biological raw materials. Thus, biomass was used for a very long time, for example in the production and processing of various wood-based materials such as cellulose, cellulose derivatives, paper, in the production of biomass-based fibers or in the production of various oilseeds or biomass materials with a high starch or sugar content (Tong et al., 2017; Marques et al. 2017; Klemm et al., 2005; Woodings, 2001). Biomass has also been used for energy purposes for a very long time, primarily for heating, but also in the production of charcoal, electricity, bioethanol, etc. (Lewis, 1981; Rodrigue, 2020), and offers an extraordinary potential as a substitute for fossil fuels (Perišić al., 2022). According to the Renewable energy statistics there were over 13.7 million renewable energy-related jobs worldwide in 2022 (Statistics, 2023a). Currently, with a renewable energy installation capacity of about 1,161 gigawatts, China is the leading country in the world, far ahead of other countries including the US, which is in second place with an installed capacity of 352 gigawatts (Statistics, 2023b).

Bioeconomy and the modern use of biomass, as its constituent element, implies the production of various materials based on biomass, the so-called bioproducts, as well as the production of advanced biofuels, especially transport biofuels (bioethanol, biogas, Fischer-Tropsch fuels, biodiesel, biohydrogen, etc.). In this sense, the technologies for the production of various biofuels, i.e., energy from biomass in general (bioenergy), are integrated with the technologies of bioproduct production into a unique concept of biorefineries. Biorefineries are used to produce energy in combined heat and power plants and biofuels, along with the production of chemicals and all with reduced or minimal negative impacts on the environment.

According to the definition given by the U.S. National Renewable Energy Laboratory (NREL), a biorefinery represents a plant that integrates biomass conversion processes and technologies in the production of fuels, energy, and chemicals (Kamm et al., 2007). A similar definition is given by the International Energy Association (IEA), according to which a biorefinery represents the sustainable processing of biomass into a number of marketable products (food, raw materials, materials, chemicals) and energy (fuels, electricity, heat) (IEA, 2014a). So, it can be said that biorefineries are similar to petrochemical refineries in which different chemical products and energy are obtained from oil as feedstock, while biorefineries use biomass as feedstock to produce various industrial products. This includes large amounts of transport fuels, e.g., biodiesel and bioethanol as products of relatively lower value, and small amounts of special chemicals or products of higher value. Some types of biorefineries may also include the production of food for human and animal consumption (Clark, Deswarte, 2015). To ensure the sustainable use of biomass, an approach has been developed that allows the highest value product to be obtained first, then the second highest, and so on (Celiktas et al. 2017).

In general, the limited fossil fuel resources as well as the constant increase of their price are the triggers for the development of the bioeconomy, in which biorefineries, as a counterpart to existing oil refineries, are a necessary element that enables the sustainability of overall production. In this regard, Kamm et al. (Kamm et al., 2016) cite the need for a gradual transition of a large part of the global economy to a sustainable economy based on biological principles, with biofuels, bioenergy and biomaterials as its supporting pillars. Also, the new situation with the Russian-Ukraine crises poses new struggles worldwide, in regard to supply chains, resource insecurities, energy poverty, etc. (Benton et al. 2022). Therefore, each country needs to ensure independent energy resources; reduce dependence and application of fossil fuels and conventional methods for energy production. Sustainable bioeconomy strategies support the achievement of some of the United Nations Sustainable Development Goals and open up new opportunities for innovation, job creation etc.(D'Amico, et al., 2022; Calicioglu, et al., 2021)

The purpose of this paper is, on the one hand, to provide additional explanations and insight into "bio-related" things that often intersect and complement each other, such as biomass, biofuel, bioenergy, biorefinery and bioeconomy.

Also, the purpose of this paper is to give a brief overview of some recent studies in the above-mentioned areas and to refer the reader to the importance of both local and global applications of the bioeconomy.

Therefore, this paper explains the origin, importance and potential of biomass as an integral element of the bioeconomy, current consumption and future projections in the context of a global perspective and current policies in this sector. It also analyzes different aspects of the origin and production of biofuels depending on the availability of different types of raw materials. Special attention is given to contemporary challenges of consumption and production, in which the bioeconomy with all its constituent elements, namely the sustainable use of biomass, the production of biofuels and raw materials, and biorefineries appears as a necessary mechanism for achieving sustainability.

BASIC TERMS AND DEFINITIONS

The bioeconomy, as a broader concept of the entire economy based on biological/renewable materials, emerges as a consequence of the long-term development of the concept of sustainable and knowledge-based progress. As with any other new sector of the economy, especially one that involves the transformation of society, understanding the appropriate progress of the bioeconomy is a challenge, primarily due to the lack of appropriate international/common databases, publications summarizing impact measurements, but also certain definitions (Schieb et al., 2015). Considering that the concept of bioeconomy is still in the development phase, there are a number of definitions that describe the term bioeconomy (Lago et al., 2019). The European Commission originally defined the bioeconomy as an economy in which renewable resources from land, sea, agriculture, fisheries and related public goods are used efficiently and sustainably to produce food, raw materials, fibers, bio-based products and bioenergy (EC, 2012). This definition was later somewhat modified to include efficient and sustainable production and processing to meet industry requirements and consumer needs, while taking into account environmental challenges, such as climate change (EC, 2018a). The bioeconomy in the United States is defined in a similar way. Thus, according to one paper, which refers to the White House National Bioeconomy Plan, it is stated that it is an economy based on the use of research and innovation in the biological sciences to create economic activity and public benefit. Furthermore, it is added that the American bioeconomy is all around us: new drugs and diagnostics to improve human health, high yields of food crops, new biofuels to reduce dependence on fossil fuel/oil and various chemical and other biologically based raw materials (Youmatter, 2020). Also interesting is the definition according to Serban, who defines bioeconomy as "the science of the dynamic integration of humanity into the environment". The same author, in the context of bioeconomy, cites the integration of economics and biology, the activities of studying market dynamics through the perspective of evolutionary biology, as well as a set of economic activities designed to optimize the production and use of biological products (Paşnicuet al., 2019).

It can be said that the bioeconomy covers a set of economic activities related to the innovation, development, production and use of biological products and processes, which will lead to significant benefits in the future in terms of improving agricultural production, increasing industrial productivity while increasing sustainability and improving public health (OECD, 2009). According to the European Bureau for Conservation and Development, the bioeconomy has the potential to mitigate climate change, between 1 billion and 2.5 billion tonnes of carbon dioxide equivalents per year by 2030 (EBCD, 2015).

Biomass is often used to obtain energy, and in this sense biomass is usually defined as any material of plant and animal origin that can be used for energy purposes. In this context, EU legislation defines biomass as a biodegradable part of products, waste and residues from agriculture (including vegetal and animal substances), forestry and related industries, as well as the biodegradable part of industrial and municipal waste (Directive 2009/28EC). In the context of the bioeconomy, the term biomass refers to renewable biological materials that are used as raw materials in the conversion into value-added products, such as food, various materials, chemicals or energy. In this case, biomass includes both edible (food) and non-edible biomass derived from plants, animals or waste streams (Sánchez et al., 2019). It is therefore important to avoid partial definitions, such as definitions that focus exclusively on the energy use of biomass or purely biological definitions, but biomass should be viewed in a broader context. From a biological point of view, biomass is defined as the total mass of living organisms, including plants, animals and microorganisms, or from a biochemical perspective, the total mass of cellulose, lignin, sugar, fat and protein materials in a given specific area (Houghton, 2008).

Some authors divide biomass into eight categories according to common, or similar, methods of measuring and determining their potential (Rosillo-Calle et al., 2007). However, biomass is often classified in one of the following categories (Bajapai 2022; Sivabalan et al. 2021; Goyal et al., 2006):

- Wood biomass - waste and residues from forestry and the wood industry, fast-growing trees (e.g., willows, poplars, eucalyptus), waste wood from other activities and wood generated as a by-product in agriculture;
- Non-wood biomass - waste, residues and by-products from the cultivation of various plants (e.g., corn, straw, cobs, stalks, shells, seeds, etc.), biomass obtained from the cultivation of oilseeds, plants rich in sugar and starch and various algae and grasses (so-called energy crops) and biodegradable part of municipal and industrial waste;
- Animal biomass - waste and residues from livestock or farms (animal faeces, mat, carcasses, etc.).

Given the wide spectrum of raw materials involved, it is impossible to speak of a typical biomass composition. For example, wood and woody plants and their residues are mainly composed of cellulose, hemicellulose and lignin in varying percentages, and are often referred to as lignocellulosic biomass (Figure 1). On the other hand, manure is rich in proteins, while cereals are rich in starch. The different chemical compositions of different types of biomass determine their different chemical properties (Tursi, 2011). Nevertheless, it can be said that biomass consists of carbohydrates, lignin, proteins, fats, oils in varying proportions, with the presence of numerous substances such as vitamins, pigments, aromas and aromatic essences.

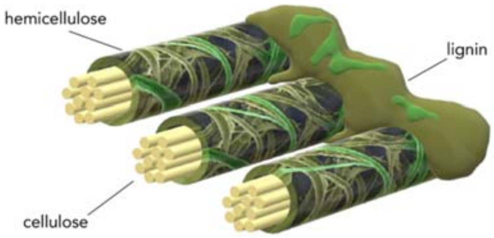

Figure 1. Complex structure of lignocelullosic biomass (Tursi, 2011)

Non-wood biomass, compared to wood biomass, is widely available, and has a more open structure, which makes it easier and cheaper to process compared to woody biomass. Also, the less energy-intensive production of bioethanol is often mentioned as an advantage of non-wood biomass. Non-wood biomass includes various plant and agricultural residues, with non-wood fibers. Some of the agricultural residues, whose bioethanol production capacities have been specifically investigated, are: corn, cassava residues, cereal straw, sugar cane residues, potato peel and oil palm biomass (Mohapatra et al., 2019; Pandey et al., 2000).

Biofuels are fuels produced directly or indirectly from biomass (Directive 2009/28EC). The main reason for processing biomass into various types of solid, liquid and gaseous fuels is to obtain fuels with a higher energy density compared to unprocessed raw biomass, and to facilitate storage and transport (Vukić and Papuga, 2014). Primary biofuels are unprocessed biomass, such as firewood, wood chips, briquettes, pellets, while secondary biofuels are fuels obtained by processing biomass, such as bioethanol, biodiesel, dimethyl ether, etc. (Nizami, et al., 2016; FAO, 2004). Biofuels are often classified from the point of view of the origin of the biomass from which they are produced into first, second and third generation biofuels (Janda and Banes, 2022; Preradovic et al. 2021; Nizami et al., 2016;), and more recently into fourth generation biofuels (Hoyos-Sebá et al. 2024; Seay and You, 2016).

Biomass is the largest renewable energy source, so the energy obtained from biomass is defined as bioenergy. It can be said that bioenergy is the energy that is contained in the organic matter of biomass. Essentially, it is the energy of the Sun that is "stored" in biomass during its biological growth, and through the process of photosynthesis (Sánchez et al., 2019; Sharma and Arya, 2019).

The total use of biomass for energy purposes is fundamentally difficult to measure, especially since most biomass is not involved in commercial transactions. However, in 2017, biomass energy is estimated to have accounted for 70% of the total renewable energy used that year, globally. However, it is important to note that most of the biomass energy relates to the traditional use for cooking and heating in developing regions. Thus, it is estimated that 86% of the primary energy of biomass in 2017, was used in the form of primary solid biofuels, including wood chips, wood pellets, and as an energy source for cooking and heating, and that only 7% of biomass energy was used as liquid biofuel (WBA, 2019).

BIOFUEL TECHNOLOGIES

The great diversity of biomass-based raw materials and products requires a wide range of different approaches and technologies for their processing. Some technologies have been present in the industry for a long time, but are also in the development phase. These advanced technologies enable the conversion of biomass into various forms of secondary energy, including electricity, gaseous and liquid biofuels, but also into various chemicals. All these technologies, or the corresponding processes, can be divided into three groups in principle (Papadokonstantakis and Johnsson, 2017; Kaltschmitt, 2017; Tursi 2011

- Thermochemical conversions (carbonization, gasification, pyrolysis),
- Physicochemical conversions (transesterification, pressing, extraction, etc.),
- Biochemical conversions (alcoholic fermentation, enzymatic hydrolysis, anaerobic fermentation, composting, etc.).

These processes yield biofuels in the form of solids (mainly charcoal), liquids (mainly alcohol and biodiesel) or gases (mainly mixtures with methane or carbon monoxide), and as schematically presented in Fig. 2. the resulting biofuels can be used for a wide range of applications, including transport or various high-temperature industrial processes.

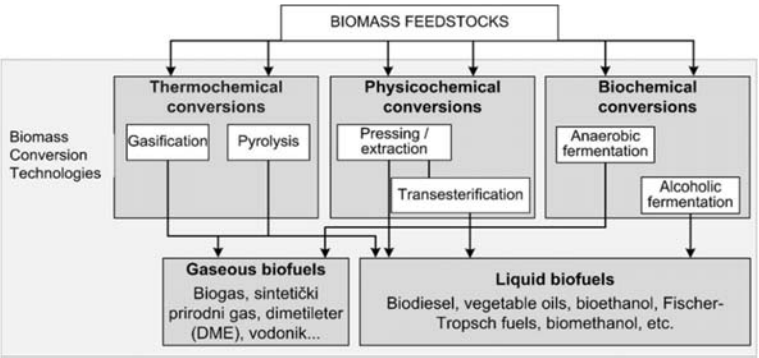

Figure 2. Different biomass conversion technologies, based on (Kaltschmitt, 2017)

Thermochemical conversions

Thermochemical processes of biomass processing include those transformation processes that are primarily caused by the action of heat, and under different process conditions (temperature, presence or absence of an oxidizing atmosphere or gasifying agent, etc.), (Vukić, Papuga, 2014). These include the processes of gasification, pyrolysis, and carbonization and, more recently, torrefaction processes. Usually, fuels produced by thermochemical processes are called synthetic biofuels. The most promising liquid synthetic biofuels, also called BtL (biomass-to-liquids), are biomethanol and Fischer-Tropsch fuels. Gaseous synthetic biofuels include dimethylether (DME) and Bio-SNG. Bio-SNG is also a form of biomethane and can be used in a similar way as a natural gas substitute, such as biogas. Alternatively, the cleaned and conditioned product gas can be converted into hydrogen. (Kumar et al. 2022; da Rosa and Ordóñez, 2022).

Among thermochemical conversions, the production of synthetic gas (syngas) by gasification of biomass is distinguished. Synthetic gas is primarily a mixture of carbon monoxide and hydrogen, but also contains smaller amounts of carbon dioxide, methane, water and other by-products, as well as nitrogen, which depends on the process conditions, the type of raw material and the performance of the gasification system. Although syngas can be used as a stand-alone fuel, its energy density is approximately half that of natural gas. Therefore, synthetic gas is mainly used as an intermediate block of molecules, for the production of transport fuels and other chemical products (Capodaglio and Bolognesi, 2021).

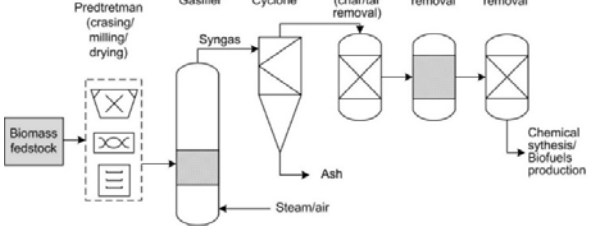

Figure 3. Syngas cleaning for downstream applications

Figure 3 shows the basic stages of synthesis gas purification used for chemical syntheses, for example for Fisher tropsh syntheses. Cyclone separators are used to remove floating particles, as well as different types of filters, such as bag filters made of textile materials. Sulfur compounds, ammonia and tar are removed using wet scrubbers. Despite the existence of various synthesis gas purification technologies, the commercial application of gasification on a large scale is still challenging, primarily due to the presence of numerous pollutants in the synthesis gas (Lotfi et al. 2021).

One of the possibilities of using cleaned synthesis gas is in the fermentation process, in the production of alcohol. The process itself is extremely challenging, and significant research efforts are being invested in the further implementation of these processes (Ellacuriaga, 2023).

Torrefaction has gained increasing attention recent years, as it produces the solid biofuels with improved properties (durability, grindability, bulk density, calorific value and energy density) compared to the untreated biomass. The process takes place under inert conditions, whereby oxygen is being removed, moisture reduced and the chemical composition changed (Olugbade and Ojo, 2020 10.1007/s12155-020-10138-3).

In general, the production of synthetic biofuels and new materials from biomass is a wide subject. Among the different processes, a special focus is on pyrolysis, which is a widely used technology and one of the most promising for the synthetic fuel production (Gvero et al., 2017).

Pyrolysis as a chemical recycling technique for plastic materials is attracting an increasing interest as an environmentally and economically acceptable option for the processing waste materials. Studies of these processes are carried out under different experimental conditions, in different types of reactors and with different raw materials (Papuga et al., 2016; Gvero et al., 2016; Papuga et al., 2013; Papuga et al. 2022; Gutierreza et al. 2022). In general, a review of recent studies on thermochemical conversion of the biomass could be find in numerous papers (Ambaye et al. 2021; Jha et al. 2022; Gonzalez and Roug, 2019; Zhang et al. 2010).

Physicochemical conversions

Physicochemical transformations of biomass include transesterification, pressing and extraction processes, which are processes used to produce high-density biofuels primarily biodiesel from triglycerides. A detailed overview of recent technologies on biofuel production from triglycerides is given by Long et al. (2021).

Basically, these processes convert different types of animal fats and vegetable oils, or triglycerides, into fuels that are similar to diesel fuel in terms of their physicochemical parameters. The transesterification process can be realized in different ways. A typical transesterification technology is shown in the process diagram in Figure 4.

Pressing and extraction processes release oils from plant raw materials, while chemical reactions of transesterification convert triglycerides with methanol or ethanol into methyl or ethyl esters, or biodiesel. These reactions take place in the presence of alkaline catalysts (KaOH or NaOH). A co-product of biodiesel production is glycerol, which can be used as a starting material for the production of a range of value-added compounds for various industries such as the food and pharmaceutical industries (ETIP bioenergy 2024; Tursi, 2019).

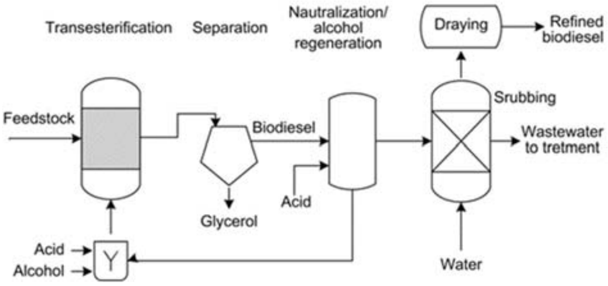

Figure 4. Process diagram of typical biodiesel production technology

Biochemical conversions

Biochemical conversions involve the transformation of biomass by microorganisms, such as yeasts, bacteria, etc. This type of biomass conversion can be processed into biofuels, which can be both liquid (e.g. bioethanol and biomethanol) and gaseous (e.g. biogas as a product of anaerobic digestion). Most bioethanol is still produced from sugar raw materials (the simplest process), but the problem of food shortage is becoming increasingly pronounced , so the development of bioethanol production is mainly directed towards the production of inedible, lignocellulosic. The main limiting factor in the use of this raw material is the complexity of its processing, due to the complexity of its structure.

Biomass needs to be subjected to a certain pre-treatment process. The production of bioethanol from lignocellulosic raw materials passes through four basic steps (Figure 5): pre-treatment, which has the task of preparing the lignocellulosic raw material for the next process; enzymatic hydrolysis, during which the polymers of lignocellulosic raw materials are converted into fermentable sugars; fermentation of sugars formed by enzymatic hydrolysis of lignocellulosic polysaccharides using appropriate microorganisms into ethanol; purification and concentration of ethanol by distillation (Balat, 2011).

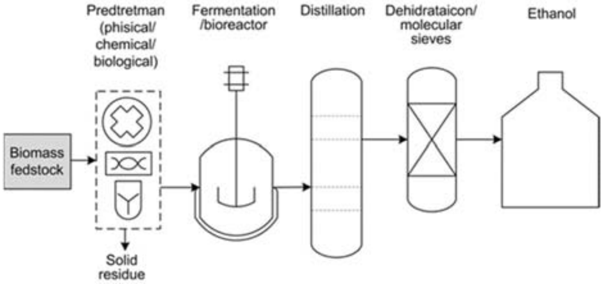

Figure 5. Process diagram of celullosic ethanol production

The enzymatic hydrolysis required to convert lignocellulose into ethanol is an expensive and technically challenging process. Today, various physical, chemical, and molecular-biological methods are available to modify the side chains of proteins in enzymes, thereby achieving more efficient use of biomass. It is expected that the widespread application of these methods will further advance this field (Fülöp et al. 2020). Recently, a series of researches have been carried out in an attempt to obtain an ideal microorganism that will be able to produce ethanol directly from any carbohydrate (Bušić, et al. 2018). The sustainability of cellulosic ethanol depends on the cost involved in each step of the bioconversion process (Devi et al., 2022).

Table 1. Review of the biomass conversion technologies

Type of the conversion technology	Classification of technologies	Reference
Thermo-chemical	Gasification	Ibarra-Gonzalez et al. 2019, Shahabuddina et al. 2020, Kirubakaran et al. 2007, Kumar et al. 2022
	Pyrolysis	Demirbas & Arin 2002, Djurdjevic et al. 2024, Wang et al. 2020, Amenaghawon et al. 2021
	Torrefaction	Preradovic et al. 2023, Djurdjevic & Papuga 2023, Olugbade & Ojo 2020, Chen et al. 2021
	Carbonization	Qin et al. 2022, Amer & Elwardany 2020, Samaniego et al. 2022
Physico-chemical	Transesterification	Vasaki et al. 2022, Hamza et al. 2020, Singh et al. 2022, Karpagam et al. 2021
	Pressing & Extraction	Show et al. 2020, Armenta et al. 2023
Biochemical	Alcohol fermentation	Kang & Lee 2015, Hwang et al. 2016,
	Anaerobic digestion	Prasad et al. 2017, Guiot & Frigon 2012, Song et al. 2015
	Enzymatic hydrolysis	Vasic et al. 2021, Santos et al. 2012, Saini et al. 2022
	Composting	Dutta & Kumar 2021, Vakili et al. 2015

Adjusting the optimal enzyme and substrate concentrations is crucial from the point of view of future industrial application. Low enzyme and substrate concentrations result in low concentrations of the obtained sugars and significantly prolong the time required to achieve a satisfactory degree of hydrolysis. On the other hand, high substrate concentrations allow the processing of larger amounts of biomass during a single cycle, but can lead to reduced hydrolysis yields due to problems related to diffusion limits caused by reduced of water content and problems in achieving homogeneity of the enzyme-substrate mixture (Kristensen, 2009; Ivetić, 2012). Recent advances in the processing of lignocellulosic biomass from agricultural waste were presented by Mujtaba et al. (Mujtaba et al. 2023). Table 1. provides an overview of the recent literature references representing all types of biomass conversion technologies.

GLOBAL POLICY AND PERPSECTIVES

The energy and material need of human society will reach a critical point in the near future. This will be primarily due to the rising costs and demand for fossil resources on which we have become dependent in terms of energy, fuels, materials and chemicals. The world's population continues to grow, and development is unprecedented in our recent history, especially in areas that have traditionally had very low demand for fossil resources (OECD, 2009). In proportion to these growing demands, it has become apparent that continuous greenhouse gas emissions and ozone depletion are affecting the global climate (De Bhowmick et al., 2018). The main strategy proposed for reducing dependence on fossil raw materials, as well as mitigating the effects of climate change, i.e., reducing carbon dioxide emissions, is the greater and more efficient use of biomass (Wiloso et al., 2012). There are three main reasons why biomass is considered an extremely important raw material. First, it is a renewable resource that could be sustainably managed in the future. Second, biomass is also considered an environmentally friendly resource. Namely, the amounts of carbon dioxide that plants absorb during their life through photosynthesis and the amounts that are released during their thermal decomposition are approximately equal, so biomass is assumed to be a “CO_2 or carbon neutral fuel” (WBA, 2012). Also, biomass has a negligible content of sulphur, nitrogen and ash, which results in lower emissions of SO_2, NOx, and soot compared to conventional fossil fuels (Zhang et al., 2007; Strehler, 2000). Third, it seems to have significant economic potential, given that fossil fuel prices will inevitably rise in the future (Demirbas and Demirbas, 2010). All this leads to the conclusion that biomass provides a positive solution for safer and more environmentally friendly production of renewable energy, including heat, electricity, and transport fuels, which can reduce emissions of carbon dioxide, sulphur and heavy metals into the atmosphere, while potentially improving rural incomes and energy security by replacing coal, oil and natural gas.

The international bioenergy market is expected to have a wide range of suppliers from several regions of the world, and bioenergy will not be affected by geopolitical issues such as oil or natural gas. Noticeable climate changes as well as environmental regulations encourage the accelerated development of the use of energy from renewable sources in meeting all energy needs, including the use of biofuels in the transport sector. Also, other legislation, such as the Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH EC 1907/2006), will lead countries to reassess the feasibility of using biomass as a feedstock for chemical production using different biotechnologies. Although this is a European example (REACH regulation), it is expected that similar regulations will affect producers and consumers worldwide (IEA, 2014b).

In 2012, the European Commission adopted the document: "Innovation for Sustainable Growth: A Bioeconomy for Europe", which presents the EU Strategy and Action Plan for the Bioeconomy. According to the aforementioned document, the orientation of the European economy towards greater use of renewable resources is defined, while respecting the principle of sustainability (EC, 2012). Thus, the document clearly states that Europe must fundamentally change its approach to the production, consumption, processing, storage, recycling and disposal of biological resources, in order to be able to manage a growing global population, the rapid depletion of many resources, increasing pressures on the environment and climate change. The strategy emphasizes that the cross-cutting nature of the bioeconomy offers a unique opportunity to comprehensively solution of interrelated societal challenges, and identifies five goals to which the strategy and action plan should contribute:

1. Ensuring food safety,
2. Sustainable management of natural resources,
3. Reducing dependence on non-renewable resources,
4. Climate change mitigation and adaptation, and
5. Creating jobs and maintaining EU competitiveness.

Three basic fields of action have been defined for the implementation of the strategies, (Vidović, 2012):

1. Investments in research, innovation and training. This includes national and EU bioeconomy funds, national funds, private investment and enhancing synergies with other initiatives.
2. Market development and competitiveness in the bioeconomy sector by sustainably increasing primary production, transforming waste streams (products) into value-added products, while simultaneous learning about the mechanisms for improved production and more efficient use of raw materials. As an example, the costs related to food waste cost taxpayers in Europe between 55 and 90 Euros per tonne of waste, resulting in170 million tonnes of CO_2. This waste can be converted into bioenergy or other bio-based products, creating jobs and economic growth at the same time.
3. Strengthening the strategy of coordination and commitment of interested participants by organizing panels on the bioeconomy, monitoring the bioeconomy and organizing conferences of interested participants.

It is clear that there are different EU policies dealing with one or more of the above objectives, which cover the fields of action of the Strategy, such as: 7th Environment Action Program, Energy Union Strategy, Forest Strategy (Forest Strategy), Circular Economy Package, Common Agricultural Policy (CAP), etc.

In 2017, a review of the achieved goals envisaged by the Strategy was performed. The review concluded that the European Bioeconomy Strategy has largely met its objectives in recent years, through a wide range of actions, from the EU Framework Programs for Research and Innovation to the launch of a public-private partnership of bio-based industries (BBI JU, Bio-based Industries Joint Undertaking), which has led to the creation of several national bioeconomy strategies, dedicated regional platforms and stakeholder panels of interested sides, which promote the development of local bioeconomies by valuing local resources tailored to local needs (EC, 2017). The BBI JU is a €3.7 billion public-private partnership between the European Union and the Bio-based Industries Consortium BIC. Until today, the BBI JU has funded 123 bio-based innovation projects involving 924 beneficiaries from 37 EU Member States and associated countries (BBI JU, 2021).

In 2018, the European Commission updated the existing strategy with the aim of accelerating the implementation of a sustainable European bioeconomy by maximizing its contribution to the 2030 Agenda and its sustainable development goals, as well as to the Paris Agreement (EC, 2018a). The update of the existing strategy makes certain adjustments to the new European policy priorities, in particular with regard to the industrial policy strategy, the circular economy and innovation in the clean energy sector, all of which emphasize the importance of a sustainable circular bioeconomy to achieve their goals. This update proposes an action plan with 14 concrete measures to be launched in 2019, based on three key priorities:

1. Strengthen and scale up organic-based sectors . This implies launching a €100 million thematic investment platform for the circular bioeconomy to bring innovations in the bioeconomy sector closer to the market and make them less risky for private investments. This also includes the development of new sustainable biorefineries across Europe, as well as the promotion and development of various standards, labelling and acceptance of bio-based products, such as the EU Ecolabel.
2. Rapidly expanding the bioeconomy across Europe. This includes various strategic programs for sustainable agriculture, forestry, food and organic production, as well as supporting bioeconomy innovation through pilot activities in rural, coastal and urban areas. Support is also provided to EU member states to develop and implement their own bioeconomy strategies.
3. Respecting the ecological limits of the bioeconomy. This includes implementing systems to monitor progress towards a sustainable and circular bioeconomy, and providing some guidance on how best to manage the bioeconomy while respecting ecological boundaries.

The European Council asked the European Commission to report on progress in implementation the EU 2018 Bioeconomy Strategy. The Progress Report identified that the actions are on track in accomplishing the main goals of the Bioeconomy Strategy, i.e., a lot of national and regional bioeconomy strategies built up cross-sectoral cooperation and sustainability regulations, as well as investing in the bioeconomy. Central and Eastern European countries have made great achievements in advancing the bioeconomy, mainly due to the substantial contributions from EU funding and the creation of new platforms and networks for collaboration. There is also, a visible increase in the investments, research and innovations in the food and bio-based industries, which shows promising developments. On the other side, this Report also identified gaps in the Bioeconomy Action Plan. First, better land management is needed, and biomass demands must meet environmental and economic needs, aligning with the goal of achieving a climate-neutral Europe. And, second, more efforts are required to promote more sustainable consumption to improve environmental integrity.

So far, the European Union has supported the bioeconomy by funding research and innovation in this sector. €3.85 billion has been invested under Horizon 2020 (2014-2020) and a further €10 billion has been provided for projects involving natural resources, including the bioeconomy, and under Horizon Europe 2021-2027, (EC, 2018b).

There are large differences in policy goals and measures supporting the bioeconomy between individual countries, which is mainly determined by the dominant industrial and economic profiles of different countries, as well as the amount of resources (biomass) at their disposal. The way in which different countries approach this issue also varies greatly. Countries such as Germany, Japan, or the United States have adopted comprehensive and coordinated bioeconomy strategies, involving numerous government bodies dealing with the environment, agriculture, research, and economy, etc. (US WHO, 2012; BBF, 2011). Other countries, such as Italy or Canada, rely primarily on industrial or regional initiatives and limit themselves to creating framework conditions at the national level (Dieckhoff et al., 2015). The United States and Canada have large forest areas, long coastlines, and arable land. Both countries have traditionally engaged in the bioeconomy on a large scale, in terms of agricultural and forestry production. However, it is recognized that new technologies can further increase the value of the agricultural and forestry sectors, while promoting rural development. In countries with few natural resources and a strong industrial structure, such as Germany, Japan, France and Italy, the bioeconomy is viewed much more in terms of its innovative potential, and, more recently, its potential for an “industrial renaissance” (Dieckhoff et al. 2015).

Unlike North America, the EU does not classify medical and biotechnological innovations as part of the bioeconomy. Its focus is first on the replacement of fossil fuels and the associated reduction of greenhouse gases, and then on achieving technological advantages using new biomass processing methods to obtain new products. In countries with scarce resources, access to and use of "alternative biomass", such as waste or other residues, play a significant role. To ensure access to raw materials, Germany, Japan, and the United Kingdom are also trying to establish international technological and resource partnerships with developing countries, which have abundant biomass reserves (Dieckhoff et al., 2015).

The appropriate use of biomass can also be a driving force for the development of relatively underdeveloped countries, which have significant resources of biomass and other renewable energy sources, as is the case in Bosnia and Herzegovina (B&H)..Unfortunately, bioeconomy projects in the Southeast European countries have been co-founded on a much smaller scale than in North-Western Europe (Lovrić et al. 2020, Lovrić et al. 2021).

It is easy to show that small municipalities in B&H (with 10,000 to 20,000 inhabitants) with a centralized wood processing industry can satisfy all their energy needs from their own wood waste, on the other hand in the production of the new technologies, because “flash” pyrolysis or other thermochemical processes can activate new sustainable economic activities in the some specific local areas (Gvero et al., 2010). The increased deployment of modern biomass-based systems, as a reliable and affordable source of energy, could be part of the solution to overcome the current constraints concerning GDP growth in Bosnia and Herzegovina (Petrović et al., 2012).

Also, proper use and better management of biomass in developing countries has significant potential to reduce existing greenhouse gas emissions from the waste management, agriculture and energy sectors (Papuga et al., 2016a; Papuga et al., 2016b).

SUSTAINABILITY AND BIOECONOMY

In the context of sustainability, the bioeconomy can also be observed as a segment of the circular economy, which can convert biological waste into valuable resources, and create innovations and incentives that will help traders and consumers reduce food waste by 50% by 2030. (EC, 2018a). Innovations in the livestock sector are increasingly enabling the safe conversion of food waste into animal feed. It is estimated that land currently used only for animal feed could, with certain innovations, feed three billion people. Cities are seen as key hubs for the bioeconomy, delivering significant economic and environmental benefits through circular urban development plans e.g. the city of Amsterdam estimates that better recycling of high-value organic waste could generate €150 million per year, create 1,200 new jobs in the long run and save 600.000 tonnes of carbon dioxide per year (EC, 2018a).

However, it should be emphasized that the transition to a modern bioeconomy is not simple a matter of mastering efficient production technologies and markets for new bio-based products, but also implies challenges such as biomass sustainability, biomass efficiency and the economics of biomass mobilization. Biomass sustainability, i.e., the sustainable supply of biomass as a raw material, is one of the key challenges for the transition to a bio-based economy. Therefore, the source of the resource should be identified from a supply and demand perspective. The biomass that can be used is of very heterogeneous origin, whether it is purpose-grown crops or residues of various crops intended for food production, as well as forest residues or seaweed. Also, municipal waste, manure and other raw materials of animal origin are considered potential resources for biologically based products and services (UNIDO and SEI, 2005). A key step in understanding the sustainability of the bioeconomy is the assessment of biomass potential of, i.e., the total amount of biomass that can be sustainably managed.

Different studies of biomass potential have mainly focused on available biomass for the bioenergy sector, often overlooking the fact that it is the same feedstock that is increasingly used in the growing production sector of biomaterials industry (Brosowski et al., 2016; Karlsson, 2014; Chum et al., 2011; Berndes et al., 2003). The assessment of the energy potential of biomass is relatively complex, and the results can vary significantly, depending on the assumptions adopted, e.g., regarding agricultural yields and trends in food requirements, the methodology for assessing the potential, or the way in which the sustainability of biomass production is taken into account and certain social and political conditions are considered (Carlson, 2014; Chum et al., 2011; Kampman et al., 2010; Haberl et al., 2010). Thus, one paper analyses various studies of biomass potential, and shows how the possible contributions of biomass in the future global energy supply range from 100 EJ/year to over 400 EJ/year in the projected year 2050, with the highest estimated potential nine times greater than the lowest estimated potential (Berndes et al., 2003). For comparison, current global primary energy consumption is approximately 50 EJ/year (Bauen et al., 2009). The main reason for such differences is that the data for the two most important parameters in the assessment of biomass potential, namely the availability of arable land and the level of yield in the production of energy crops, are very unreliable and subject to different interpretations. Modern biomass use, as an integral part of the bioeconomy, combined with traditional uses such as food, energy, building materials, raw materials for pulp and paper, etc. will increase the pressure on existing biomass resources. Therefore, efficient use of available biomass resources is crucial to meet future requirements for biomass (Brosowski et al., 2006; Karlsson, 2014).

The current expansion of the biomass energy industry, accompanied by rising commodity prices, is raising public concern about the imposing a choice of land use , of which the areas are certainly limited, between use for food, raw materials or energy production. The impact of biofuels on food prices is attracting increasing attention. Industries that are strictly tied to biomass as a raw material, such as the pulp and paper industry, question policies that promote the production of energy from biomass (bioenergy), arguing that priority in the use of biomass should be given to the production of materials rather than energy (Hoeltinger, 2012). However, currently less than 1% of agricultural land globally is used for energy purposes, primarily for cereals, sugarcane, oilseeds and palm trees, which accounts for a relatively small part of the total land-use change associated with other activities. Perhaps the most significant impact of bioenergy on land use is related to the way forest management has changed in countries with large forest industries and where the use of biomass for energy has increased significantly, such as Finland and Sweden (Berndes et al., 2016).

The development of what are called "first generation biofuels", i.e., fuels produced from edible agricultural products, was a necessary step in advancing the technology into a more sustainable and environmentally friendly system. The biggest problem with first-generation biofuels is their competitiveness with food production, and the consequent increase in food prices (Khattar et al., 2016). This problem is partly solved by promoting second-generation biofuels, i.e., fuels derived from the inedible part of agricultural crops (e.g., flakes, ears, corn, straw, pruning residues), generally inedible biomass (e.g., different types of grass, waste) or from purpose-grown tree crops, inedible oilseeds, etc., i.e., from energy crops. However, in the general case, the raw material is more complex compared to the raw materials for first-generation biofuels, and therefore more sophisticated and complex for processing and production equipment is required (Nizami et al., 2016).

A special problem is the extraction of useful sugars from lignin and cellulose, and the need for appropriate enzymes to release sugar molecules from cellulose. In general, cellulosic ethanol currently costs two to three times more than the energy-equivalent amount of fossil fuel (Carriquiry et al., 2011). Third-generation biofuels, i.e., algae-derived biofuels, are considered to be an extremely promising approach in the biofuels sector (Dragone et al., 2010; Alam et al., 2015). Algae production is not competitive with food production, and a special advantage is the very high production in comparison to traditional agricultural crops. While traditional crops are harvested once or twice a year, depending on the culture and climate, microalgae can be harvested of every 10 to 30 days, which significantly increases the total yield of biofuels derived from algae (Schenk et al., 2008). A fairly high potential for carbon dioxide binding to useful biomass is often reported (Zhao and Su, 2014; Bai et al., 2017). Recently, there has been a lot of talk about fourth-generation biofuels, which relate to the use of genetically modified algae that have higher biofuel production (Shokravi et. al., 2022; Abdullah et al., 2019; Seay and You, 2016; Dutta et al., 2014). It is predicted that in the near future, the main sources of biodiesel will be third- and fourth- generation biofuels (Lugani et al., 2019). According to IEA projections, by 2035 these advanced biofuels will account for 20% of total global biofuel production (Seay and You, 2016).

CONCLUSION

Bioeconomy as a broader concept of the whole economy based on biological/renewable materials appears as a response to various challenges, such as climate change, resource scarcity and rapid population growth, that are imposed on modern civilization. Biomass as the resource on which the bioeconomy is based is today the only known renewable resource that can provide a number of positive solutions in the context of the mentioned challenges. Various studies show that the potential of biomass is extremely large and that exceeds the current needs of humanity, but this does not mean that it should be exploited to its fullest extent. The use of biomass should be viewed from the perspective of supply and demand, while respecting the limits of sustainability. An important segment of the bioeconomy is the production of various fuels from biomass, the so-called biofuels. Since biomass is a raw material of very heterogeneous composition and origin, there are different technologies for its processing into biofuels and various chemicals that can be used as raw materials in industry. A broad overview of these technologies is provided in this paper.

The increase in biofuel production also imposes challenges such as competitive use of land for biomass and food production. Currently, less than 1% of agricultural land globally is used for energy purposes, primarily cereals, sugar cane, oilseeds and palm crops, which makes a relatively small part compared to the total land use change associated with other activities. Also, if we take into account the negative effects of the use of fossil fuels, as well as certain chemicals and materials on the environment, we come to the inevitable conclusion that new production systems must be developed, based on renewable raw materials. Globally, the only such source of renewable raw material is biomass.

Recognizing the above issues, many countries have adopted their national bioeconomy strategies, such as the United States, Canada, Germany, Japan, including the European Union as a community of states. In 2012, the European Commission adopted the document: "Innovation for Sustainable Growth: Bioeconomy for Europe", which represents the EU strategy and action plan for the bioeconomy, which defines the orientation of the European economy towards greater use of renewable resources while respecting the principles of sustainability. In 2018, the European Commission updated the existing strategy with the aim of accelerating the implementation of a sustainable European bioeconomy, and maximizing its contribution to the 2030 Agenda and its sustainable development goals, as well as to the Paris Agreement. So far, the European Union has supported the bioeconomy through funding research and innovations in this sector. About €3.85 billion has been invested under Horizon 2020 (2014-2020) and a further €10 billion has been secured for projects involving natural resources, including the bioeconomy, and under Horizon Europe 2021-2027).

Given the previous considerations, it is clear that there is a global interest in research and investment in the biomass and biofuel supply chain, which gives an optimistic picture regarding the future use of biomass as the foundation of the future global bioeconomy.

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Summary/Sažetak

Bioekonomija kao širi pojam cjelokupne ekonomije zasnovane na biološkim/obnovljivim materijalima se javlja kao posljedica suočavanja sa brojnim globalnim izazovima današnje civilizacije. Ovaj rad objašnjava nastanak ovakvog koncepta, sa posebnim osvrtom na njegove sastavne elemente, odnosno održivo korišćenje biomase i proizvodnju biogoriva. Dat je pregled globalnih politika i strategija u ovoj oblasti, kao i potencijalno korišćenje biomase. Razne studije pokazuju da postoji ogroman potencijal biomase, koji prevazilazi trenutne potrebe čovječanstva. Međutim, korišćenje biomase treba posmatrati sa tri aspekta, odnosno, sa aspekta ponude, potražnje i održivosti. Povećanje proizvodnje biogoriva nameće i izazove kao što je konkurentno korišćenje zemljišta za proizvodnju biomase i hrane, što je poseban problem, pa su u tom pogledu razvijene različite tehnologije koje koriste nejestivu biomasu u proizvodnju biogoriva i vrijednih hemikalija. Prepoznajući značaj bioekonomije, kao neizbježnog elementa u postizanju održivog razvoja, Evropska Unija je 2012. godine usvojila strategiju i akcioni plan za bioekonomiju. Takođe, mnoge zemlje koje predstavljaju važne igrače u globalnoj ekonomiji, kao npr. Sjedinjene Američke Države, Njemačka, Kanada, Japan, itd., usvojile su svoje nacionalne strategije koje promovišu bioekonomiju. Jasno je da postoji globalni interes za istraživanje i ulaganje u lance snadbijevanja biomase i biogoriva, što daje optimističnu sliku u pogledu buduće upotrebe biomase kao osnove buduće globalne bioekonomije.

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New insight into the geochemical behavior of alkali and alkaline earth metals in a frequently subsampled clay pit sample with and without Al-normalization

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Abstract: Clays are fine-particle-size materials (less than 2 μm) comprised mainly of clay minerals, which are hydrated aluminum silicates with associated alkali and alkaline earth elements. The small particle size and complex porous structure result in a high specific surface area, which allows strong physical and chemical interactions with dissolved species. Metals can also be incorporated within the clay structure, which can be made of octahedral and/or tetrahedral sheets. Depending on the composition of the tetrahedral and octahedral sheets, the layer will have no charge or will have a net negative charge. If the layers are charged, this charge is balanced by interlayer cations. The recent research of metals in clays mainly focused on heavy metals, while investigations of light metals are rather rare. For the investigation of the behavior of alkali and alkaline earth metals in clay samples, 110 subsamples of cored samples were taken. The total contents of potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), strontium (Sr), and barium (Ba) were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES). Descriptive statistics were conducted using maximum, minimum, average, and median values. Correlation and PCA analysis with and without Al-normalization were calculated to determine the different behavior of metals, The contents of metals lie in the following sequence: K>Mg>Ca>Na>Ba>Sr. The highest correlation between metals was found between Mg and K (r=0.87) and Ba and Sr (r=0.65). PCA analysis revealed two subgroups: 1) K, Mg, Na. 2) Ba, Sr. After the Al normalization was applied, Ba and Sr showed the highest correlation (r=0.81). Ca did not significantly contribute to any of the PCA subgroups, suggesting a possible different source and behavior.

INTRODUCTION

Fine-grained clays are naturally occurring materials, found in silt, rocks, and soil. According to Pinnavaia (1983), they are mostly composed of hydrous aluminosilicates, which make up the majority of the colloidal fraction (particle size below 2 m) of rocks, sediments, and soil. The small particle size, complex and porous structure, and high specific surface area of clays and clay minerals enable robust physical and chemical interactions with dissolved species (Uddin, 2017). Clays are abundant, and the clay-based minerals are derived from versatile raw materials (Aboudi Mana et al., 2017). The layered structure of the clay minerals oversees several chemical and physical characteristics. Understanding the intricate clay mineral structure and the mechanisms that keep ions and molecules attached to their surface is challenging (Cygan et al., 2009). The mineralogy and reactive character of clays are the main factors that influence their behavior and ion adsorption kinetics. The adsorption properties are mostly controlled by the surface area of the clay minerals and the porosity of the interlayer (Deng et al., 2017). Clays are particularly good at removing cations, or heavy metals, from solutions due to their high cation exchange capacity (Edi et al., 2015). The surface activity of clays is controlled by various factors, including their chemical composition, the type of exchangeable cations present, the charge of the layer, and the character of the surface atoms (Schoonheydt and Johnston, 2012). The surface of the clay allows for the selective adsorption of various cations. One of the two cations will be more strongly attracted if both are present. The different adsorption strengths are difficult to explain. It depends on the charge, its origin and structure. The size of the ion will determine how two cations with the same charge will adsorb to each other. Water molecules are dipoles. Therefore, positively charged cations attract partially negatively charged water molecules. The hydrated cation is small because the smaller cation has a higher positive charge density. Deeper sediments, however, are more stable due to the decreased degree of change (Bauer and Velde, 2014). When it comes to determining the metal content of clays, there are several studies on the content of heavy metals (Sen Gupta & Bhattacharyya, 2012; Chen et al., 2016; Nkansah et al., 2016; Uddin, 2017), while studies on alkali and alkaline earth metals are very rare. Therefore, in this study, an analysis of alkali and alkaline earth metals was performed. Although alkali and alkaline earth metals are quite similar in chemical characteristics, their behavior in clay minerals is different. Some of them (K, Rb) are easily replaced by another element, and some are mainly adsorbed on clay particle or incorporated into the interlayer space (Annabi-Bergaya et al., 2006; Arevalo, 2018). With this research, we intend to address this rarely investigated topic. It is challenging to identify which part of nature comes from which source, since metals come from both anthropogenic and natural sources. Natural sedimentary metal content can vary by several orders of magnitude based on the origin, character, and grain size of the metal-rich bedrock. A geochemical strategy is often used to address these issues, This method uses conservative elements (Al, Fe, Li, Rb, Sc) that represent one or more important metal carriers in nature (such as clay minerals, iron and manganese oxides, and organic matter) for geochemical normalization (dividing the metal content) (Covelli and Fontolan, 1997). Schropp and Windom (1988) stated that Al is one of the best markers for terrestrial materials for several reasons, including its abundance in nature, high immobility, resistance to anthropogenic influences, and resistance to diagenesis and strong redox effects in sediments that could change the metal content in sediments. Al-normalization is necessary for interpreting the source of metals (Ho et al., 2012). To enable the Al normalization, the content of all alkali and alkaline earth metals was divided by the content of aluminum. Following the formation of the ratios, the correlations between them were used. The clay content of the clay pit sediment might be less variable due to the normalisation to Al. When there are more samples, it makes more sense to statistically evaluate the correlation results. This makes the correlations more reliable. If a comparative study is based on a data set that is truly too small, which is not uncommon in the literature, its conclusions are subject to a low level of reliability (Yang et al., 2019). The aims of the study were:

a) To estimate the content of alkali and alkaline earth metals (K, Na, Mg, Ca, Ba and Sr) in 110 subsamples of the core clay pit sample.
b) To gain insight into the similarities and differences of metals in geochemical behavior and origin, through correlations and PCA analysis, with and without Al-normalization.
c) The pH in water, pH in $CaCl_2$ and electrical conductivity were measured in order to gain insight into the acidity/alkalinity of the samples and the content of water-soluble ions.

MATERIALS AND METHODS

Sampling and Samples

In Central Bosnia and Herzegovina (43° 51′ 54′′ N, 18° 13′ 36′′ E), a clay pit sediment core sample was obtained from the "Rapailo" clay deposit. The site is situated in the Sarajevo Canton, not far from the state capital Sarajevo (Figure 1). Clay in this region is mined for brick making. The area of the clay pit is 300 m wide and 1300 m long. The region with the clay deposits has a temperate continental climate. The average annual temperature in this climate zone is 12°C, with an average high of 1°C in January and an average low of 22°C in July. Precipitation averages up to 1000 mm per year.

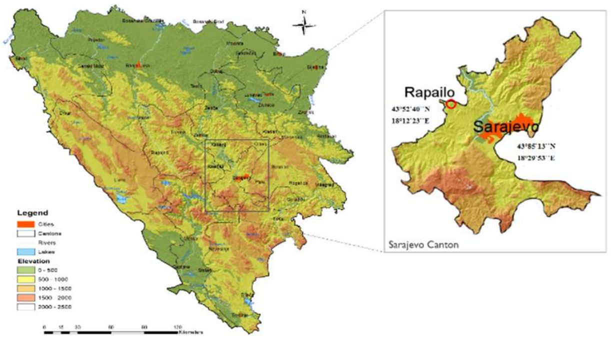

Figure 1. Bosnia and Herzegovina with sampling position (Jurković et al., 2021)

The mineral composition in order of increasing abundance is shown in Table 1.

Table 1. Mineral composition of the Rapailo clay samples (Kapetanović, 2004)

Mineral	Chemical formula
quartz	SiO_2
illite, 2M2	$K_{0.7}Al_{2.1}(SiAl)_4O_{10}(OH)_2$
illite, 2M	$(K, H_3O)Al_2Si_3AlO_{10}(OH)_2$
kaolinite	$Al_2Si_2O_5(OH)_4$
muscovite, 3T	$KAl_2(Si_3Al)O_{10}(OH)_2$
muscovite, 2M1	$KAl_2(Si_3Al)O_{10}(OH, F)_2$
muscovite, 2M2	$(K, Ca)Al_2(SiAl)_4O_{10}(OH)_2$
chlorite, chrome	$(Mg, Cr)_6(SiAl)_4O_{10}(OH)_8$
chlorite	$(Mg, Fe)_6(SiAl)_4O_{10}(OH)_8$

The clay deposits of the Sarajevo basin are part of the sedimentary genetic types that are created by the decomposition and leaching of all types of aluminosilicate rocks. The source material was always somewhat distant from the process of mechanical redeposition. Due to the sorting, which is most often expressed in the form of layers, water was used as a transport medium. Rapid variations in the type of material and grain size can also be seen in lenticular structures. In Bosnia and Herzegovina, clay deposits were primarily formed during the Quaternary and Neogene under external conditions. Further evidence that allochthonous clays exist in fully humid conditions comes from their close association with the production of coal deposits. The geological map of the research area is shown in Figure 2. (Jovanović et al., 1978).

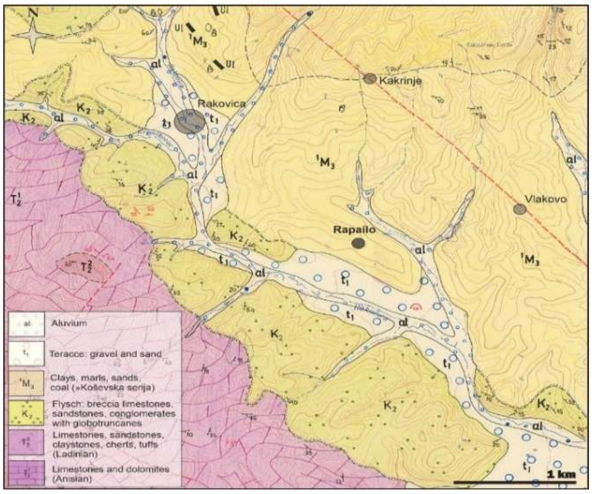

Figure 2. Geological map of research area(Jovanović et al., 1978)

A 33 m-long clay mine core sample was extracted (Figure 3). The core sample was divided into subsamples on site. A separate subsample was extracted from the core sample very frequently (every 30 centimeters). By dividing the sample into subsamples, 110 subsamples were obtained. Five hours after extraction, the samples were packed in sterile polypropylene bags and sent to the laboratory. The samples were transported at 4°C in a refrigerator. The subsamples, or cores, exhibited a unique spectrum of colors at the bottom of the sediment, which included off-white, pale grey, dark black, orange, and dark grey (Figure 3).

Figure 3. Motor-operated sediment corer and samples

Sample preparation

The clay pit core samples were homogenized, allowed to air dry, and then sieved through a 2-mm sieve. Teflon tubes were filled with approximately 0.2 g (accuracy of 0.001 g) of the sample. Concentrated hydrofluoric acid and concentrated nitric acid were then added. The samples were then prepared in forty tubes using microwave digestion. Three microwave digestion sessions were required to process all 110 samples. Under conditions of pressure of 55 bars and a constant temperature increase from 25 to 230°C, the digestion process lasted three hours.

Methods of Analysis

Basic quality parameters

To assess the acidity/alkalinity and soluble ions amount, pH in H_2O (pH_W), and $CaCl_2$ (pH_{Ca}) and electrical conductivity (EC) were measured., The pH of the core samples was determined using a Mettler Toledo MP 220 device. The instrument was calibrated with buffers with a pH value of 4.21 and 7.00. The clay extraction solution ratio was 1:5 (10 g clay and 50 mL extraction solution), according to ref 31. The measurement was performed with two different extraction solutions: ultrapure water and calcium chloride (0.01 mol/L).

Electrical Conductivity (EC), was measured in the claypit sample/ultrapure water (1:5) ratio, with a Mettler Toledo MC 126 instrument. Using the procedure described in the EPA 9050 approach, the instrument was calibrated with the standards of known EC.

Metals

The total of elements content was determined using optical emission spectrophotometry with inductively coupled plasma. Agilent 7000 ICP-OES apparatus was used to perform measurements. In this way, the total contents of K, Na, Mg, Ca, Ba and Sr were analyzed. The standards of known contents of the investigated elements were utilized for measurement. To prevent excitation interference, 1% CsCl was added to all samples used for iron measurement. The analytical results were in good agreement with the reference materials used (sandy loam and BAM 112 a),

Statistical evaluation

The results of the study were statistically evaluated using SPSS 20.0 SPSS Inc., Chicago, IL and United States Statistical Programs.

Descriptive Analysis: Descriptive statistics were presented as maximum, minimum, mean, average, and median value, along with standard deviation (SD) and coefficient of variance (CV). Standard deviations and coefficients of variance were used to show the distribution of heavy metals (Yongming et al., 2006).

Correlation: The degree of similarity between two sets of results can be measured by correlation. The following is a description of the correlation coefficients, which indicate how strongly two variables are related: (0.0–0.19), weak connection (0.2–0.39), moderate connection (0.40%–0.59), strong connection (0.60%–0.79), and extremely strong connection (0.80%–1.0). When the correlation coefficient (r) is positive, the connection is uphill; when it is negative, the correlation is downhill.

The correlation between the results for heavy metals was also normalized, as it has been shown to be one of the best indicators of terrigenous material, in order to take into account for the different proportions of clay mineral fractions. (Brown et al., 2000).

Principal component analysis (PCA): PCA is very often used in statistical evaluation in environmental studies (Díaz et al., 2002). In order to reduce the data sets and obtain a smaller number of recorded variables or PCs, PCA is commonly applied. An eigenvalue >1 was the cutoff point for retains of PCs, indicating that these PCs accounted for more variance than was accounted by one of the original data sets. These variables are used to analyze the relationship between variables (Loska and Wiechuła, 2003). If there is a high variability in the correlations and standard deviations of the variables, the PCA results will also vary. (Farnham et al., 2003). PCA is applied to the correlation matrix if there are significant differences in order of magnitude and each parameter makes an equal contribution. The VARIMAX normalized rotation method, where the maximized variation of the factor loadings is carried out, can be used to better understand multivariate analysis. The factor loadings between 0.71 and 0.32 are considered very good, while those below 0.32 are regarded extremely poor. (Yongming et al., 2006).

RESULTS AND DISCUSSION

Basic quality parameters (pH_w, pH_{ca}, EC) and descriptive statistics parameters (average, max, min, median, SD, CV) for the determined alkali and alkaline earth metals are presented in Tables 2 and 3. Vertical distributions of the measured elements are shown in Figures 4 to 9.

Table 2. Basic quality parameters

	pH_w	pH_{Ca}	ΔpH	EC (mS/cm)
Average	6.61±0.16	6.09±0.15	0.5	3.1±0.1
Maximum	8.50	7.69	0.9	16.0
Minimum	3.72	3.63	0.0	0.5
Median	6.42	5.09	0.6	1.9
SD	1.14	0.97	0.2	3.2
CV, %	17.2	16.0	43.0	102.9

	Average (mg/kg)	Max. (mg/kg)	Min. (mg/kg)	Median (mg/kg)	SD (%)	CV (%)
K	19655.2±786.2	33253.2	3181.6	19834.3	7325.8	37.3
Mg	8069.4±484.64	15702.9	1561.4	8233.8	3070.1	38.0
Ca	3629.5±108.9	15360.0	739.1	2816.6	2544.2	70.1
Na	756.0±37.8	2892.8	181.8	710.8	349.6	46.2
Sr	76.7±2.3	400.4	23.8	49.1	76.2	104.1
Ba	44.6±1.3	267.4	7.6	76.7	37.7	84.6

Table 3. Alkali and alkaline earth metals determination

Vertical distributions of measured elements:

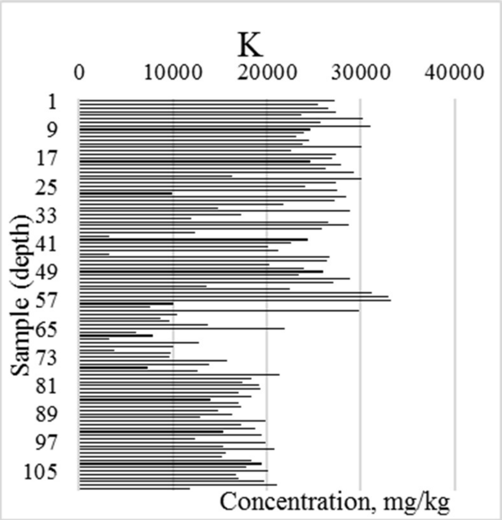

Figure 4. Vertical distribution of K

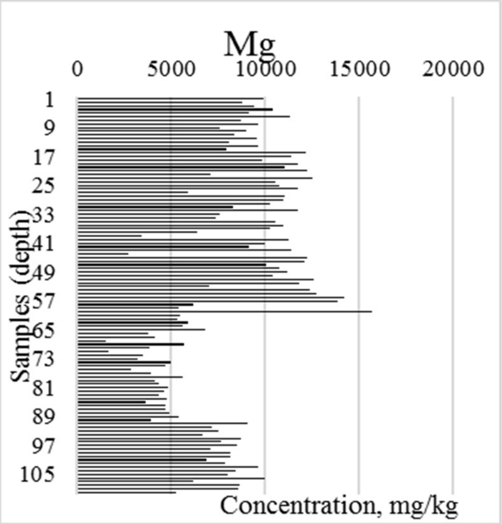

Figure 5. Vertical distribution of Mg

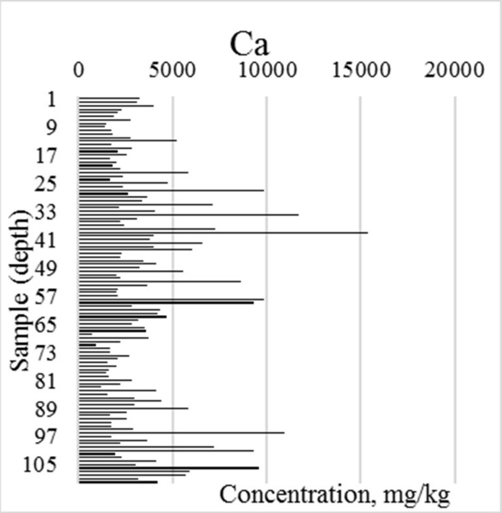

Figure 6. Vertical distribution of Ca

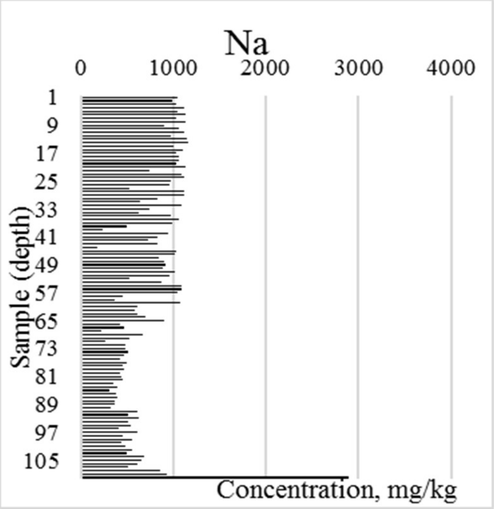

Figure 7. Vertical distribution of Na

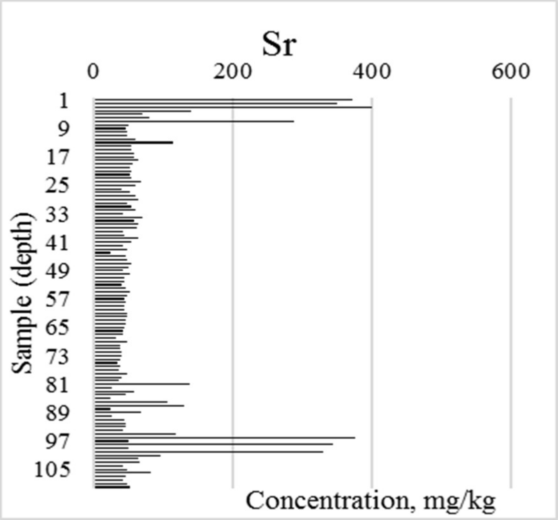

Figure 8. Vertical distribution of Sr

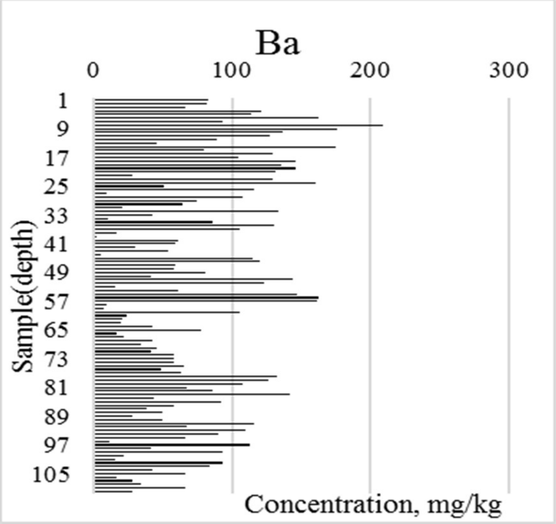

Figure 9. Vertical distribution of Ba

The pH (in H_2O and $CaCl_2$) varied greatly among samples. pH increased with sample depth. The highest pH value was found in sample 96 (8.50 and 7.69), and lowest in the sample 39 (3.72 and 3.63). Alkaline pH promotes metals precipitation and adsorption on the clay surface (Lukman et al., 2013). Characteristics of metals adsorption differ for different clay minerals (Farrah & Pickering, 1978). Natural clay is a mixture of different minerals which makes it difficult to say the exact reason for adsorption of metals. EC was higher in the upper parts of the cored sample. The highest value was found in sample number 42 (16.0 mS/cm). The minimum value was found in the sample 106 (0.5 mS/cm). Higher EC means a higher amount of easily desorbed ions (metals) from the clay particle (Choo et al., 2022).

Of all the metals investigated, K had the highest abundance (0.32%–3.33%). The average content was 1.96%. Very comparable results were found by Cherian et al. (2018). The sample 68 (3181.6 mg/kg) had the lowest content of K in the middle portion of the sediment (depth ranged from 19.30 to 19.60 m). K content was highest in sample 57 (33253.2 mg/kg) (depth ranged from 16.00 to 16.30 m). K content shows a gradual downcore decrease. The lowest CV was found in the case of K, meaning that the K variability in content is lowest throughout the sediment core. Sample 60 (1502.9 mg/kg) had the highest Mg content (depth from 16.90 to 17.20 m). Less Mg was found in deeper sediment layers, according to the vertical distribution of Mg. In sample 69 (1561.4 mg/kg), the Mg measurement yielded the lowest content (depth from 19.30 to 19.60 m). The average Mg content was comparable to that of a recent study (Diko-Makia and Ligege, 2020). The maximum Ca content was found in the upper parts of the clay sediment in sample 39 (15360.0 mg/kg) (depth from 11.40 to 11.70 m). The minimum content was found in the sample 68 (739.1 mg/kg) (depth from 19.30 to 19.60 m). The average amount of Ca was similar to the study of Cherian et al. (2018). The Ca content varied greatly throughout the sediment. Up to about 20 meters below the surface, the Na content was quite uniform. The average Na content was 756.04 mg/kg. The middle portions of the clay sediment, sample 44 (181.8 mg/kg), had the lowest Na content (depth from 12.10 to 12.40 m). The last sample (110) (2892.8 mg/kg) had the highest content (depth range: 32.10 to 32.40 m). The average Na content in the soils is slightly lower than that found in the study by Williams et al. (2008). Sample 3 (400.4 mg/kg) had the highest Sr content (depth from 0.6 to 0.9 m). Less Sr was found in the middle sediment layers, according to the vertical distribution of Sr. In sample 85 (23.8 mg/kg), the Sr measurement yielded the lowest content (depth from 24.40 to 24.70 m). The average Sr content was lower than in a recent study (Mohamed et al., 2022). The highest CV was found for Sr, meaning that the variability of Sr content was the highest throughout the sediment core. Ba had the lowest average content of all the metals under investigation (7.63 mg/kg-267.39 mg/kg). The average content was 44.60 mg/kg. The amounts of Ba found in the clay samples were lower than average soil content (>19 mg/kg) (Kabata-Pendias, 2010). Sample 69 (7.6 mg/kg) had the lowest amounts of Ba in the middle portion of the sediment (depth ranged from 19.30 to 19.60 m). The Ba content was highest in sample 14 (267.4 mg/kg) (depth ranged from 3.90 to 4.20 m). Ba showed the lowest standard deviation of all measured metals. The minimal contents of K, Mg, and Ca were found in sample 68, (depth ranged from 19.30 to 19.60 m).

Correlations

Correlations among measured metals without Al-Normalization are presented in Table 4.

Table 4. Correlations among metals without Al-normalization

	Mg	Na	K	Ca	Ba	Sr
Mg	1.00					
Na	0.63	1.00				
K	0.87	0.64	1.00			
Ca	-0.15	-0.24	-0.41	1.00		
Ba	0.24	0.22	0.21	0.26	1.00	
Sr	0.09	0.05	0.12	0.16	0.65	1.00

High correlation coefficients indicate similar geochemical behavior of the elements. A very strong correlation was observed between K and total Mg (r=0.87). This high correlation is a result of the fact that K and Mg are constituents of aluminosilicate materials, especially chlorite, which is found in this clay pit. The strong correlation between Sr and Ba (r=0.65) can be explained by their similarity. Both metals are alkaline earth elements, with the same valence state and similar ionic radii (Ba^{2+} - 149 pm, Sr^{2+} - 132 pm with coordination number 6, and Ba^{2+} - 156 pm, Sr^{2+} - 140 pm with coordination number 8) (Luther III, 2016). Since they are members of group II A elements, their behavior is representative of the interaction of alkali-earth elements with clays and soil fractions (Hatipoglu et al., 1990). Strong and positive correlations were also found in the case of K and Na, which are very similar in behavior and characteristics. Na can be found in the interlayers of various clays containing K. (Kumari and Mohan, 2021). A strong correlation (r=0.63) was found between Na and Mg, and the reason for this correlation could be the fact that Na can replace K in the mineral structure or adsorb on clay particles in a similar way. It is significant that Ca showed only negative correlations with other metals, indicating that Ca behaves differently and has different origin.

Correlations among metals with Al-normalization

The Al normalization was used to minimize the effect of the fluctuating clay mineral content. The results of correlation coefficients after Al normalizations are shown in Table 5.

Table 5. Correlations with applied Al-Normalization

	Mg	Na	K	Ca	Ba	Sr
Mg	1.00					
Na	0.48	1.00				
K	0.75	0.44	1.00			
Ca	0.49	0.16	0.04	1.00		
Ba	0.45	0.24	0.32	0.29	1.00	
Sr	0.47	0.21	0.38	0.27	0.81	1.00

After normalization, Sr showed an even higher correlation with Ba than without Al-normalization (r=0.81). The Ba^{2+} ion is bound to different clay minerals. In kaolinite, Ba^{2+} ion sorption occurs mainly at surfaces and edges. In the case of chlorite-illite mixed clay and montmorillonite, appreciable amounts of Ba^{2+} ion are sorbed between the layers. Sorption is reversible in kaolinite, and only partially reversible in chlorite-illite and montmorillonite (Eylem et al., 1990). The correlation between Mg and K is slightly reduced (r=0.75). Ca, which showed only negative correlations before Al normalization, now shows low but positive correlations. It also shows a moderate correlation with Mg (r=0.49). This result may indicate that Ca is found in some other minerals beside clay minerals in this clay pit, for example, calcite or dolomite.

PCA Analysis

Varimax rotation was used for principal component analysis (PCA). The metal content (K, Mg, Ca, Na, Sr, and Ba) was employed as possible variables, both with and without previously completed normalization.

3.3.1. PCA analysis without Al-Normalization

The six main components detected by monitoring the total amount of the metals under examination without normalization comprised 100.00% of the experiment's overall variance, according to an analysis of the key components using oblimin rotation (Table 6). The experiment's total variability was comprised of 73.429% of the first two major components. Table 6. shows the principal components' eigenvalues, the percentage of variance, and the cumulative variance.

Table 6. Total variance without Al-Normalization

	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	2.669	44.482	44.482
2	1.737	28.947	73.429
3	0.768	12.806	86.236
4	0.443	7.376	93.612
5	0.304	5.061	98.672
6	0.080	1.328	100.000

The most significant variables for PC1 were the contents of K, Mg, and Na with contents of eigenvectors (loadings) greater than 0.8 and a significant positive result; for PC2, the most significant variables are the content of Ba and Sr with similarly high contents of eigenvectors greater than 0.8, according to the indicators of the relationship between the main components and the analyzed variables (Table 7).

Table 7. Component matrix without Al-Normalization

	Component	
	1	2
Mg	0.895	-0.076
Na	0.809	-0.115
K	0.933	-0.178
Ca	-0.339	0.615
Ba	0.400	0.813
Sr	0.261	0.805

A two-dimensional graph showing the spatial distribution and mutual interaction of the investigated variables (properties) was created utilizing the principal components (PC1 and PC2) as the spatial dimensions (Figure 10). In the graphical presentation, vectors stand in for the examined variables. Graph 10 shows that K, Mg, and Na are grouped in a very small area of the graph, indicating a high similarity in behavior. Sr and Ba are also similar, but Ca behaves differently from the other metals.

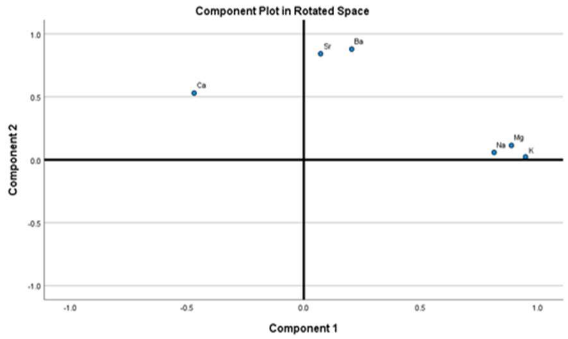

Figure 10. Component plot in rotated space without Al-Normalization

Kleven and Alstad (1996) found that in montmorillonite-$CaCl_2$ systems, a moderate selectivity for Ca^{2+} ions over Na^+ was observed. Also, a higher affinity for Ba^{2+} over Ca^{2+} was found in the clays. Spatially separated metals may indicate that their sources are different from each other.

PCA analysis with Al-normalization

By analysis of the main components using oblimin rotation, the six main components obtained by monitoring the total content of the analyzed metals with normalization contained 100.00% of the total variance of the experiment (Table 8). The first two main components contained 69.331% of the total variability of the experiment.

Table 8. Total variance with Al-Normalization

Initial Eigenvalues		
Total	% of Variance	Cumulative %
3.026	50.441	50.441
1.133	18.890	69.331
0.927	15.454	84.785
0.605	10.087	94.873
0.188	3.135	98.008
0.120	1.992	100.000

The indicators of the relationship between the main components and the analyzed variables (Table 9.) show that the most significant variables for PC1 were the content of Mg with contents of eigenvectors (loadings) greater than 0.8 with a significant positive result, while the content of K, Ba, and Sr also has high eigenvectors (greater than 0.7).

Table 9. Component matrix with Al-Normalization

	Component	
	1	2
Mg	0.873	-0.243
Na	0.578	-0.522
K	0.725	-0.501
Ca	0.485	0.326
Ba	0.759	0.484
Sr	0.771	0.458

By using the main components (PC1 and PC2) as spatial dimensions, a two-dimensional graph was constructed showing the spatial distribution and mutual relationship of the analyzed variables (properties). The analyzed variables are represented by vectors on the graphic display. From Figure 11 it can be seen that K and Na, on one side and Ba and Sr are on the other, which is similar to the version without Al-normalization. When we use Al normalization, Ca and Mg show different characteristics. When we use Al-normalization, Mg and Ca are separated from the other elements, which may indicate that they have a different source. The short-term clay-lime interaction involves the adsorption of free Ca ions onto clay minerals (Cherian et al., 2018).

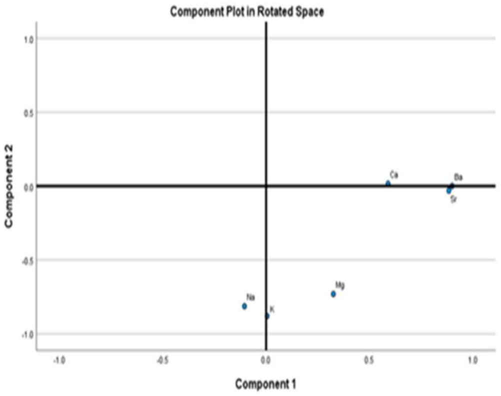

Figure 11. Component plot in rotated space with Al-Normalization

CONCLUSIONS

The present study focused on evaluating the behavior of alkali and alkali earth metals (K, Na, Mg, Ca, Sr, and Ba), with and without the application of Al normalization. The results were evaluated using correlations and PCA analysis. From the results obtained it can be concluded that:

- The investigated metal contents lie in the following sequence: K>Mg>Ca>Na>Ba>Sr.
- The metal pairs: Sr and Ba; K and Na, K and Mg show similar geochemical behavior and origin.
- Ca showed almost no similarity to other metals when Al-normalization is applied, indicating that Ca has a different behavior and origin, perhaps from carbonates.

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Summary/Sažetak

Gline su materijali sitnih čestica (veličine manje od 2 µm) koji se uglavnom sastoje od minerala gline, koji su hidratizirani aluminijski silikati s pripadajućim alkalnim i zemno alkalnim metalima. Mala veličina čestica i složena porozna struktura rezultiraju visokom specifičnom površinom, što omogućuje snažne fizičke i kemijske interakcije s otopljenim vrstama. Metali se također mogu ugraditi u strukturu gline, koja može biti izrađena od oktaedarskih i/ili tetraedarskih ploča. Ovisno o sastavu tetraedarskih i oktaedarskih ploča, sloj neće imati naboj ili će imati ukupni negativni naboj. Ako su slojevi nabijeni, ovaj naboj se uravnotežuje međuslojnim kationima. Novija istraživanja metala u glinama uglavnom odgovaraju teškim metalima, dok su istraživanja lakih metala prilično rijetka. Za ispitivanje ponašanja alkalnih i zemno alkalnih metala u uzorcima gline uzeto je 110 poduzoraka sondiranog uzorka. Ukupni sadržaj kalija (K), magnezija (Mg), kalcija (Ca), natrija (Na), stroncija (Sr) i barija (Ba) određen je pomoću induktivno spregnute plazme optičke emisijske spektroskopije (ICP-OES). Deskriptivna statistika provedena je pomoću maksimalnih, minimalnih, prosječnih i vrijednosti medijane. Za određivanje različitog ponašanja metala, izračunata je korelacija i PCA analiza sa i bez Al-normalizacije. Sadržaj metala je u sljedećem nizu: K>Mg>Ca>Na>Ba>Sr. Najveća korelacija između metala utvrđena je između Mg i K (r=0,87) te Ba i Sr (r=0,65). Multivarijantnom statističkom analizom izdvojene su dvije grupe: 1) K, Mg, Na. 2) Ba, Sr. Nakon primjene Al normalizacije, Ba i Sr pokazuju najveću korelaciju (r =0,81). Ca nije značajno pridonio ni jednoj grupi PCA, što ukazuje na mogući drugačiji izvor i ponašanje.

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Antioxidant Properties of Alien Plant Species: Assessing Phenolic and Flavonoid Content, Antioxidant, Oxidant Capacities, and Antimicrobial Activity

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Abstract: Alien plant species pose significant threats to natural ecosystems due to their adaptability and invasive potential. In this study, the antioxidant activity of 13 alien and invasive plant species was investigated, regarding their total phenolic content, flavonoid content, total antioxidant capacity, total oxidant capacity, and antimicrobial activity. Plant tissues were macerated, evaporated to dryness, and the extracts were dissolved in water. Total phenolic content ranged from 6.55 mg GAE/g extract to 30.82 mg GAE/g extract. Flavonoid content spanned from 3.04 mg Quercetin/g extract to 40.81 mg Quercetin/g extract. The total antioxidant capacity ranged between 662 CEAC µM and 1540 CEAC µM. Total oxidative capacity can be correlated to the abiotic stress. *Rhus typhina* L. and *Impatiens balfourii* Hooker f., exhibited positive total oxidant capacity values of 122.56 ± 7.85 µM H_2O_2 Equiv./L and 141.38 ± 4.33 µM H_2O_2 Equiv./L, respectively. These two species also showed the highest total antioxidant capacity that were 1190,06 ± 137,36 CEAC µM for *Rhus typhina* L and 1540,34 ± 270,84 for CEAC *Impatiens balfourii* Hooker f. The results suggest that the extraction solvent significantly affects the antioxidant capacity, with ethanol generally providing higher extraction efficiency for many species. The antimicrobial activity was determined against *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Candida albicans*. *Rhus typhina* L was active against *E. coli* and *P. mirabilis*. This data indicates that plants increased antioxidant defense systems against abiotic stresses. Clearly, the correlation between total oxidant capacity and total antioxidant capacity values in plants is a valuable factor indicating the mechanism of the plant defense.

INTRODUCTION

Plants possess a robust antioxidant system that protects against oxidative stress, a condition induced by an imbalance between reactive oxygen species (ROS) production and detoxification. This system controls cellular homeostasis but may also be a biomarker for environmental stressors. Reactive oxygen species (ROS), produced in a different subcellular compartment, have dual roles—serving as signaling molecules in biological processes while potentially causing damage such as DNA alteration, membrane disruption, and cell death when their levels are uncontrolled (Apel et al., 2004; Mittler, 2002). The group of antioxidant enzymes, including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), are most important protectors against exacerbated ROS production forming a dynamic defense network to mitigate oxidative damage (Noctor et al. 1998, Foyer et al. 2005).

For example, in environments with elevated ozone (O_3) levels, plants face increased oxidative burdens, as O_3 exposure triggers increased ROS production, leading to metabolic disturbances. Both enzymatic and non-enzymatic mechanisms contribute to ROS generation, with their activity modulated by oxygen availability (Sharma et al. 2012, Veljovic-Jovanovic et al. 2006). This adaptability underlines the complexity of the plant antioxidant system, highlighting its critical role in maintaining redox balance and its potential utility as an indicator of environmental stress (Ainsworth et al. 2016, Feng et al. 2019).

The measurement of flavonoids and phenolic content in plants plays a critical role in understanding their antioxidant capacity and their response to environmental stress. Flavonoids and phenolic compounds are secondary metabolites with potent antioxidative properties, acting as ROS scavengers and metal chelators, thus contributing to the mitigation of oxidative stress. These compounds can directly neutralize ROS, reduce oxidative damage, and enhance the overall resilience of plants under stress conditions such as drought, salinity, and high ozone levels (Heim et al. 2002, Rice et al. 1997).

The accumulation of flavonoids and phenolics is often triggered by environmental factors, making their quantification a valuable biomarker for assessing plant stress levels. For instance, under ultraviolet (UV) radiation or ozone exposure, plants tend to increase the synthesis of these compounds as part of their protective strategy. Moreover, flavonoids, particularly those localized in the epidermal tissues, can act as UV filters, reducing light-induced oxidative damage. Similarly, phenolic compounds play a role in strengthening the plant cell wall and modulating enzyme activities associated with stress response pathways (Agati et al. 2010, Nicholson et al. 1992).

Total antioxidant capacity (TAC) represents the overall ability of the plants to neutralize reactive oxygen species (ROS) and prevent oxidative damage. It encompasses the combined effects of all antioxidant molecules, including enzymatic antioxidants (e.g., superoxide dismutase, catalase, and peroxidase), non-enzymatic antioxidants (e.g., ascorbic acid, glutathione, and carotenoids), and secondary metabolites like flavonoids and phenolics (Apel et al. 2004, Rice-Evans et al. 1997). On the other hand, flavonoid and phenolic content specifically quantify the presence of these particular secondary metabolites, which are important but not the sole contributors to antioxidant defense (Heim et al. 2002, Sharma et al. 2012).

Flavonoids and phenolics play a significant role in scavenging free radicals and chelating metal ions, directly mitigating oxidative stress. However, enzymatic antioxidants react with specific ROS in distinct subcellular compartments, and non-phenolic compounds like ascorbate and tocopherols give specific antioxidant functions that flavonoids and phenolics cannot fully substitute (Foyer et al. 2005, Stalikas, 2018). However, during severe oxidative stress, enzymatic antioxidants may play a more dominant role by regenerating non-enzymatic antioxidants and repairing damaged molecules (Noctor et al. 1998).

Alien (non-native, introduced, exotic) plant species are the species that were introduced to an area in which they were never naturally distributed, by intentional or unintentional anthropogenic activities (Mitić et al., 2008). Many of them have adapted to the conditions of the new habitat and become a danger to natural ecosystems by invading the habitats of native species and suppressing their growth, or human health, by causing allergies. Most of these plants are resistant to herbicides, parasites and other plant pests, probably as the result of various antimicrobial compounds they contain. However, the research of the antimicrobial and antioxidant activity of alien species has only begun to attract the attention of researchers in the last decades (e.g. Ahmad et al., 2011; Albouchi et al., 2013; Rattanata et al., 2014; Morah t al., 2015; Szewczyk et al., 2016, Aissani et al., 2018).

The aim of this research was to evaluate the total oxidative stress antioxidant capacity, and the phenolic and flavonoid content of plant species alien to the region of Bosnia and Herzegovina. Selected plants with the highest antioxidative capacity were tested for antimicrobial activity. Alien plants, often characterized by their adaptability and robust secondary metabolite production, may offer novel antioxidant agents. This research not only contributes to understanding the biochemical potential of these species but also highlights their role as a possible resource for developing new pharmaceuticals with high antioxidant capacity.

MATERIALS AND METHODS

Samples

For the purpose of this research, the following alien plant species were selected: *Rhus typhina* L. (deciduous tree of the Anacardiaceae family, native to eastern North America), *Robinia pseudoacacia* L. (deciduous tree of the Fabaceae family, native to North America), *Acer negundo* L. (deciduous tree of the Aceraceae family, native to North America), *Ailanthus altissima* (Mill.) Swingle (deciduous tree of the Simaroubaceae family, native to Asia), *Reynoutria japonica* Houtt. (herbaceous perennial of the Polygonaceae family, native to Asia), *Impatiens balfourii* Hooker f. (herbaceous perennial of the Balsaminaceae family, native to Asia), *Amaranthus albus* L. (herbaceous annual of the Amaranthaceae family, native to the tropical Americas), *Galinsoga parviflora* Cav. (herbaceous annual of the Compositae family, native to South America), *Commelina communis* L. (herbaceous annual of the Commelinaceae family, native to Asia), *Panicum capillare* L. (herbaceous annual of the Poaceae family, native to North America), *Senecio inaequidens* DC (herbaceous perennial of the Compositae family, native to Africa), and *Erigeron annuus* (L.) Pers. and *E. canadensis* L. (herbaceous annuals of the Compositae family, native to North and Central America). The plant samples (leaves for trees, all aboveground parts for herbaceous plants) were collected within the administrative boundaries of the city of Sarajevo in Septemper and October 2023, air-dried in the laboratory, powdered in a blender and kept in marked, sealed glass jars at room temperature.

Chemicals

Following chemical were used: Gallic acid, p.a. ACS reagent ≥98 %, (Carl Roth), Folin-Ciocalteu's phenol reagent, Analytical CRM (CPAchem), Sodium carbonate, puriss. p.a. ACS reagent Ph. Eur anhydrous ≥99,8 % (Sigma-Aldrich), Aluminium chloride hexahydrate, p.a. >99 %, (Centrohem), Quercetin, HPLC ≥95 % (Sigma-Aldrich), Sodium chloride, puriss. p.a. ACS reagent Ph Eur. ≥99,5 % (Honeywell), Xylenol orange sodium salt, ACS reagent (Acros Organics), Sulfuric acid, 98 %, (Merck), o – Dianisidine dihydrochloride, 98 %, (Thermoscientific), Ammonium iron (II) sulfate hexahydrate, analytical reagent grade ≥99 % (Fisher Scientific), Glycerol, analytical reagent grade ≥99 %, (Fisher Scientific), Ethanol, puriss. p.a. absolute ≥99,8 % (Honeywell). The chemicals were used as received from their original packaging without undergoing any additional purification.

Maceration extraction

Extracts of all 13 plants were prepared by weighing approximately 2.5 ± 0,01 g of dried plant material, which was then transferred to a 500 mL Erlenmeyer flask. Subsequently, 250 mL of distilled water was added, and the mixture was placed on a magnetic stirrer at 500 rpm and 60°C for 60 minutes. After the extraction, the mixture was filtered through Whatman filter paper. The filtrate was collected and evaporated to dryness in a water bath at 60°C. The extracts were weighed.

Soxhlet extraction

Extracts of all 13 plants were prepared using Soxhlet extraction. Dried plant material were weighed and transferred into an extraction thimble, which was then placed into the Soxhlet extractor. Subsequently, 150 mL of 96 % ethanol was added, and the Soxhlet extraction was performed for 3 hours. After extraction, the extracts were filtered through Whatman filter paper and evaporated to dryness in a water bath at 60°C. The extracts were then weighed.

Samples

The samples for analysis were prepared by weighing approximately 0.1 g ± 0,1 mg and dissolving it in 10 mL of 96 % ethanol and in deionized water. The following analyses were performed on these samples: total oxidant capacity, total antioxidant capacity, total phenolic content, total flavonoid content, and antimicrobial activity.

Total oxidant capacity

In this assay (Erel, 2005), oxidants in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion, a process that is enhanced by the presence of glycerol in the reaction mixture. The ferric ion then reacts with xylenol orange in an acidic medium, producing a colored complex. The intensity of the color, which can be measured using a spectrophotometer, reflects the total concentration of oxidants in the sample. The assay is calibrated using hydrogen peroxide, and results are reported in micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 Equiv./L) mean value ± SD in the Table 1. The composition of Reagents 1 and 2 is described below. For the measurement, 20 μL of the sample is mixed with 200 μL Reagent 1, and the absorbance is first measured at 560 nm. Afterward, 50 μL of Reagent 2 is added, and the absorbance is recorded again at the same wavelength after 45 minutes.

Reagent 1 contains 150 μM xylenol orange, 140 mM sodium chloride, 1.36 M glycerol, with the pH adjusted to 1.75. This solution is stable for at least 6 months when stored at 4°C. Reagent 2 includes 5 mM ferrous ammonium sulfate and 10 mM o-dianisidine dihydrochloride, and it also remains stable for a minimum of 6 months at 4°C. Both reagents are prepared in 25 mM sulfuric acid. The results are given in Table 1. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Total antioxidant capacity

Antioxidant capacity was tested by e-BQC lab instrument based on a redox potential of the tested compounds and its comparison with antioxidant activity of ascorbic acid. Measurements are expressed in charge units—micro Coulombs (μC). To compare the results (μC) to antioxidant capacity of Vitamin C Antioxidant Capacity Equivalents (CEAC) are used, and results are expressed as mean value ± SD in the Table 2. Calibration curve with a standard solution of ascorbic acid was ussed to measure charge values.

Determination of Total Flavonoid Content

The total flavonoid content was determined using the method described by Cao-Ngoc (2020), with slight modifications. A 150 μL aliquot of the sample was transferred to a microplate, followed by the addition of 150 μL of 2 % Aluminum Chloride solution. After a 10-minute incubation, the absorbance was measured. The assay was calibrated using a standard quercetin solution, and the results were expressed as mg Quercetin equivalents per gram of extract (mg QE/g), calculated as the mean value ± standard deviation (SD). The results are given in the Table 3. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Determination of Total Phenolic Content

The total phenolic content in the extracts was measured using the Folin-Ciocalteu method (Gonçalves et al., 2013), with slight modifications. A 500 μL aliquot of the extract was transferred to a 10 mL volumetric flask, followed by the addition of 500 μL of deionized water and 500 μL of Folin-Ciocalteu reagent. After 3 minutes, 1,5 mL of 20 % sodium carbonate was added, and the flasks were filled to volume with deionized water. The solutions were incubated in the dark for 45 minutes, after which the absorbance was recorded at 765 nm. Gallic acid was used as the standard, and total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). Results are presented as mean values ± standard deviation (SD) in the Table 4. Absorbances were measured in SpectraPlate-96 MB by Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Determination of Antimicrobial Activity

Luria Bertani (LB) agar was prepared by dissolving 40 g of growth media (Carl Roth, Germany) in 1 L of distilled water. LB broth was prepared by dissolving 10 g of Tryptone, 5 g of Yeast extract, and 10 g of NaCl (all Sigma-Aldrich, USA) in 1 L of distilled water. Saburaud dextrose agar (Liofilchem, Italy) was prepared by dissolving 65 g of media in 1 L of distilled water. Saburaud dextrose broth (Merck, Germany) was prepared by dissolving 30 g of media in 1 L of distilled water. All growth media were autoclaved at 121°C for 20 minutes.

Antimicrobial activity screens

Antimicrobial activity was tested against the following bacteria and a fungus: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, and Candida albicans*.

The bacterial cultures were grown for 24 h at 37°C in LB broth and Candida albicans in Saburaud dextrose broth under the same conditions. Next, the cultures were diluted in 0.9% sodium chloride solution to match a 0.5 McFarland turbidity standard. The uniform growth was obtained by streaking 4 mm thick LB agar plates three times with a Drigalski spatula (100 µL of initial inoculum), rotating the plate for 90° in-between streaks. To establish putative antimicrobial activity against Candida albicans, Saburaud dextrose agar plates were streaked. The plates were air-dried under sterile conditions for 5 minutes. Then, wells with a diameter of 8 mm were punched aseptically with a sterile cork borer. Next, ethanol and water extracts (100 µL) of plants selected for this study were introduced into the wells. The experiments were performed in triplicates. The plates were left at room temperature for 15 mins and incubated for 24 h at 37°C. Zones of inhibition were measured as the diameter of the halo around the well.

RESULTS

Total oxidant capacity

The total oxidant capacity (TOC) results (Table 1.) demonstrate that the activity of different plant species varies depending on the extraction solvent. *Rhus typhina* has similar TOC values for both water and ethanol extracts, with slightly higher activity in water, suggesting that both polar and semi-polar compounds contribute to its properties. *Impatiens balfourii* shows much higher activity in water extracts, indicating that water-soluble compounds play a key role. On the other hand, *Erigeron canadensis* displays high TOC only in ethanol extracts, indicating its active compounds are more soluble in ethanol.

Table 1. Total oxidant capacity

Species	Water extracts	Ethanol extracts
Rhus typhina	122,56 ± 7,85	111,31 ± 1,13
Erigeron annuus	/	/
Erigeron canadensis	/	164,75 ± 3,66
Impatiens balfourii	141,38 ± 4,33	70,98 ± 3,02
Amaranthus albus	/	78,85 ± 3,65
Robinia pseudoacacia	/	79,95 ± 2,00
Ailanthus altissima	/	96,20 ± 3,28
Galinsoga parviflora	/	92,08 ± 0,80
Acer negundo	/	109,77 ± 6,18
Reynoutria japonica	/	/
Commelina communis	/	88,84 ± 5,30
Panicum capillare	/	82,84 ± 3,96
Senecio inaequidens	/	185,95 ± 10,16

Other plants, such as *Amaranthus albus*, *Robinia pseudoacacia*, and *Ailanthus altissima*, exhibit moderate TOC in ethanol extracts, with *Acer negundo* having the highest among them. *Senecio inaequidens* shows the strongest overall activity in ethanol extracts. The lack of TOC values for water extracts in some species may be due to the poor solubility of their compounds in water.

Total antioxidant capacity

The results for antioxidant capacity ($\overline{X} \pm SD$ CEAC µM), given in the Table 2., show varying activities across different plant species and extraction solvents, with both water and ethanol extracts yielding distinct values. In general, ethanol extracts exhibit slightly higher antioxidant capacities than water extracts for many species.

Table 2. Total antioxidant capacity

Species	Water extracts	Ethanol extracts
Rhus typhina	1190,06 ± 137,36	797,21 ± 134,02
Erigeron annuus	1132,71 ± 22,72	1250,18 ± 88,09
Erigeron canadensis	937,21 ± 105,54	1084,31 ± 167,63
Impatiens balfourii	1540,34 ± 270,84	1250,90 ± 165,00
Amaranthus albus	662,85	1023,33 ± 108,89
Robinia pseudoacacia	820,88	1022,24 ± 70,27
Ailanthus altissima	993,58 ± 31,33	1326,40 ± 123,63
Galinsoga parviflora	730,63	1434,56 ± 166,93
Acer negundo	1003,03 ± 116,57	703,57 ± 65,23
Reynoutria japonica	1224,60 ± 230,01	1401,53 ± 32,74
Commelina communis	982,17	1131,85 ± 59,65
Panicum capillare	1097,19 ± 181,60	786,33 ± 65,48
Senecio inaequidens	971,75	1440,73 ± 79,67

Rhus typhina shows a significant difference between water (1190.06 ± 137.36 µM) and ethanol (797.21 ± 134.02 µM) extracts, with the water extract demonstrating stronger capacity. Similarly, *Erigeron annuus* displays higher activity in ethanol (1250.18 ± 88.09 µM) compared to water (1132.71 ± 22.72 µM). In contrast, *Impatiens balfourii* has a notably higher antioxidant capacity in water (1540.34 ± 270.84 µM) than in ethanol (1250.90 ± 165.00 µM).

Amaranthus albus and *Robinia pseudoacacia* show relatively low activity in water extracts (662.85 µM and 820.88 µM, respectively) compared to their ethanol counterparts (1023.33 ± 108.89 µM and 1022.24 ± 70.27 µM). This trend continues with *Ailanthus altissima*, which shows higher capacity in ethanol (1326.40 ± 123.63 µM) than in water (993.58 ± 31.33 µM). *Galinsoga parviflora* follows a similar pattern with a substantial increase in ethanol (1434.56 ± 166.93 µM) compared to water (730.63 µM).

Reynoutria japonica displays high antioxidant capacity in both extracts, with ethanol (1401.53 ± 32.74 µM) slightly exceeding water (1224.60 ± 230.01 µM). In contrast, *Acer negundo* and *Panicum capillare* show a reverse pattern, with higher activity in water (1003.03 ± 116.57 µM and 1097.19 ± 181.60 µM) than in ethanol (703.57 ± 65.23 µM and 786.33 ± 65.48 µM). Lastly, *Senecio inaequidens* shows its highest antioxidant capacity in ethanol (1440.73 ± 79.67 µM) compared to water (971.75 µM).

These results suggest that the extraction solvent significantly affects the antioxidant capacity, with ethanol generally providing higher extraction efficiency for many species, though there are notable exceptions where water extracts show superior activity.

The total phenolic content

The results for total phenolic content (TPC) (Table 3.) in different plant extracts show that water generally yields higher TPC compared to ethanol. For example, ***Erigeron canadensis*** has the highest TPC in water (30.82 ± 3.12 mg GAE/g), while ***Erigeron annuus*** also has high phenolic content in water (29.51 ± 2.65 mg GAE/g). Conversely, ***Galinsoga parviflora*** has the lowest TPC in water (6.55 ± 1.15 mg GAE/g), but its ethanol extract shows a higher value (11.75 ± 0.44 mg GAE/g).

Impatiens balfourii shows a significant difference between water (17.46 ± 0.38 mg GAE/g) and ethanol (7.33 ± 0.61 mg GAE/g), favoring water. However, ***Acer negundo*** is one of the few plants where ethanol (21.52 ± 1.54 mg GAE/g) outperforms water (18.77 ± 0.90 mg GAE/g).

Table 3. Total phenolic content

Species	Water extracts	Ethanol extracts
Rhus typhina	27,22 ± 0,97	20,93 ± 1,79
Erigeron annuus	29,51 ± 2,65	13,29 ± 0,82
Erigeron canadensis	30,82 ± 3,12	20,24 ± 2,62
Impatiens balfourii	17,46 ± 0,38	7,33 ± 0,61
Amaranthus albus	29,23 ± 2,45	12,78 ± 1,36
Robinia pseudoacacia	22,49 ± 1,62	13,05 ± 1,99
Ailanthus altissima	23,74 ± 1,11	19,69 ± 1,42
Galinsoga parviflora	6,55 ± 1,15	11,75 ± 0,44
Acer negundo	18,77 ± 0,90	21,52 ± 1,54
Reynoutria japonica	27,62 ± 0,98	16,69 ± 0,98
Commelina communis	14,07 ± 0,74	9,59 ± 2,99
Panicum capillare	23,74 ± 1,62	9,58 ± 0,74
Senecio inaequidens	17,88 ± 1,87	16,69 ± 1,03

Overall, these results suggest that water is generally a more effective solvent for phenolic extraction, but ethanol can be superior for certain species. This variability underscores the need to optimize extraction methods depending on the plant and the application, such as in antioxidant production or natural product research.

The Total Flavonoid Content

The results for total flavonoid content (TFC) (Table 4.) across water and ethanol extracts reveal distinct patterns in solvent efficiency for flavonoid extraction. In contrast to the results for phenolic content, ethanol extracts generally show higher flavonoid content compared to water extracts. For example, *Rhus typhina* shows a significant difference, with ethanol extracts yielding 65.14 ± 1.22 mg QE/g, far higher than the water extract (24.10 ± 0.84 mg QE/g). Similarly, *Impatiens balfourii* has an exceptionally high TFC in ethanol (69.41 ± 0.55 mg QE/g) compared to water (9.62 ± 2.28 mg QE/g).

Table 4. Flavonoid phenolic content

Species	Water extracts	Ethanol extracts
Rhus typhina	24,10±0,84	65,14 ± 1,22
Erigeron annuus	19,05± 2,59	18,18 ± 1,01
Erigeron canadensis	40,81±1,83	47,77 ± 2,68
Impatiens balfourii	9,62±2,28	69,41 ± 0,55
Amaranthus albus	32,02±2,88	67,24 ± 1,81
Robinia pseudoacacia	14,76±2,00	52,09 ± 5,20
Ailanthus altissima	19,78±2,64	82,18 ± 3,31
Galinsoga parviflora	4,66±0,03	57,64 ± 1,20
Acer negundo	3,04±0,03	71,80 ± 2,67
Reynoutria japonica	40,22±1,64	36,29 ± 0,78
Commelina communis	13,13±0,53	34,99 ± 1,17
Panicum capillare	22,27±1,19	57,32 ± 1,46
Senecio inaequidens	18,63±3,30	66,04 ± 2,41

Some species show more balanced results, like *Erigeron canadensis*, where both water (40.81 ± 1.83 mg QE/g) and ethanol (47.77 ± 2.68 mg QE/g) extracts contain similarly high levels of flavonoids. *Reynoutria japonica* is another outlier, where the water extract (40.22 ± 1.64 mg QE/g) slightly exceeds the ethanol extract (36.29 ± 0.78 mg QE/g), suggesting that water is more effective for flavonoid extraction in this species.

Species like *Ailanthus altissima* (82.18 ± 3.31 mg QE/g), *Acer negundo* (71.80 ± 2.67 mg QE/g), and *Amaranthus albus* (67.24 ± 1.81 mg QE/g) show significantly higher flavonoid content in ethanol, indicating that ethanol is generally the better solvent for extracting flavonoids from most of the species in this study. The variability between species and solvents highlights the importance of choosing the appropriate solvent depending on the plant and target compounds for specific applications.

Antimicrobial and fungicidal activity screens

Out of 26 plant extracts tested, only extracts corresponding to *Rhus typhina* showed antimicrobial activity. Water extracts of *Rhus typhina* inhibited the growth of *Escherichia coli* (average inhibition zone diameter 15 mm) and *Proteus mirabilis* (17 mm). Ethanol extracts of the same species inhibited the growth of *Staphylococcus aureus* (21 mm), *Proteus mirabilis* (20 mm), and *Pseudomonas aeruginosa* (19 mm). Antimicrobial activity against *Candida albicans* was not detected.

DISCUSSION

Total phenolic and total flavonoid content

The total phenolic content of *Rhus typhina* in the water extracts (27.22 ± 0.97 mg GAE/g) and ethanol extracts (20.93 ± 1.79 mg GAE/g) is consistent with values reported in the literature, such as Liu (2019), who reported a range of 25.96 ± 52.26 mg GAE/g. However, our values are notably higher than those for fruit extracts reported by Fu (2011), which ranged from 0.1188 ± 0.0011 to 5.85 ± 0.1859 mg GAE/g. Similarly, the total flavonoid content in water extracts (24.10 ± 0.84 mg QE/g) falls within the range of 15.71 to 62.53 mg QE/g reported by Wang (2015) for leaf extracts, while the ethanol extracts (65.14 ± 1.22 mg QE/g) are near the upper limit of this range. For fruit extracts, Wang (2015) found values between 11.31 to 71.46 mg QE/g. These variations may result from differences in extraction solvents, plant parts used, or environmental factors.

The total phenolic content of *Erigeron annuus* in our water extracts (29.51 ± 2.65 mg GAE/g) is lower than the range reported by Zhang (2020), which was 31.6 to 152.8 mg GAE/g, with water being the most effective solvent. Similarly, our ethanol extracts (13.29 ± 0.82 mg GAE/g) are considerably lower than the 92.6 ± 1.2 mg GAE/g reported by Zhang. This variation could be due to differences in extraction methods, geographic origin of the plant material, or environmental conditions.

For the total flavonoid content, our water extracts (19.05 ± 2.59 mg QE/g) and ethanol extracts (18.18 ± 1.01 mg QE/g) are also significantly lower than the range reported by Zhang, where flavonoid content ranged from 46.7 to 480.2 mg QE/g, with the ethanol extract reaching 26.8 ± 0.4 mg QE/g. The lower flavonoid content in our extracts could be attributed to different plant parts, solvent efficiency, or extraction techniques used.

The total phenolic content of *Erigeron canadensis* in our water extracts (30.82 ± 3.12 mg GAE/g) and ethanol extracts (20.24 ± 2.62 mg GAE/g) is significantly lower than the values reported by Barhoumi et al. (2024) and Polat et al. (2022). Barhoumi et al. reported much higher phenolic content (95.59 ± 0.4 mg GAE/g) from essential oils produced by hydrodistillation, while Polat et al. found 71.34 ± 0.53 mg GAE/g using different extraction methods. These differences highlight the impact of extraction techniques and plant material used on phenolic yield.

For total flavonoid content, water extracts (40.81 ± 1.83 mg QE/g) and ethanol extracts (47.77 ± 2.68 mg QE/g) are much lower than the 467.00 ± 0.53 mg QE/g reported by Barhoumi et al. (2024). However, our values exceed those reported by Polat et al. (2022), who found 18.91 ± 1.46 mg CA/g. The differences in results could be influenced by the use of different standards (QE vs. CA) and extraction methods, as Barhoumi et al. focused on essential oils, which may yield different concentrations of phenolics and flavonoids.

The total phenolic content of *Impatiens balfourii* in water extracts (17.46 ± 0.38 mg GAE/g) is similar to the flower extracts reported by Maleš et al. (2020), which were 18.6 ± 0.6 mg GAE/g. However, our ethanol extracts (7.33 ± 0.61 mg GAE/g) are closer to the values reported for leaf extracts (7.9 ± 0.2 mg GAE/g) by Maleš et al. This variation may be due to the different plant parts used, as we used stem extracts, which may yield different phenolic concentrations compared to leaves and flowers.

Regarding flavonoid content, our water extracts (9.62 ± 2.28 mg QE/g) and ethanol extracts (69.41 ± 0.55 mg QE/g) show higher values than the leaves and flowers reported by Maleš et al. (2020), where leaf extracts contained 4.6 ± 0.1 mg QE/g and flower extracts 20.5 ± 0.1 mg QE/g. The notably higher flavonoid content in our ethanol extracts suggests that solvent choice and plant part can significantly influence flavonoid yield.

The total phenolic content of *Amaranthus albus* is 29.23 ± 2.45 mg GAE/g in water extracts and 12.78 ± 1.36 mg GAE/g in ethanol extracts, indicating that water is a more effective solvent for extracting phenolics in this case. In contrast, the total flavonoid content is 32.02 ± 2.88 mg QE/g in water extracts and 67.24 ± 1.81 mg QE/g in ethanol extracts, suggesting that ethanol is more efficient for flavonoid extraction.

These results highlight differences in extraction efficiency depending on the solvent used. Comparing these findings with data from other *Amaranthus* species could provide a broader understanding of the variability in phytochemical content within the genus. Future research could focus on these comparisons to further elucidate the phytochemical profile and potential applications of *Amaranthus albus*.

The total phenolic content (TPC) of *Robinia pseudoacacia* in our study was measured at 22.49 ± 1.62 mg GAE/g in water extracts and 13.05 ± 1.99 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was found to be 14.76 ± 2.00 mg QE/g in water extracts and 52.09 ± 5.20 mg QE/g in ethanol extracts.

Comparatively, Stankov et al. (2018) reported a total phenolic content of 0.77 mg GAE/mL and a total flavonoid content of 0.17 mg QE/g of extract from flower extracts of *Robinia pseudoacacia.* Uzelac et al. (2023) found a significantly higher TPC of 123.38 mg GAE/g of dry weight and a TFC of 21.21 mg CE/g DW from leaf extracts.

These variations underscore the influence of the plant part utilized and the extraction method on phytochemical yield. While our findings for TPC are higher than those reported by Stankov et al., they remain lower than the values obtained by Uzelac et al. The observed higher flavonoid content in ethanol extracts suggests ethanol is more effective for flavonoid extraction in this species.

The total phenolic content (TPC) for *Ailanthus altissima* in our study was 23.74 ± 1.11 mg GAE/g in water extracts and 19.69 ± 1.42 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was measured at 19.78 ± 2.64 mg QE/g in water extracts and 82.18 ± 3.31 mg QE/g in ethanol extracts.

Comparing these findings to the literature, Luís et al. (2012) reported significantly higher TPC values in stems (44.95 ± 0.97 mg GAE/g), stalks (52.48 ± 1.69 mg GAE/g), and leaves (136.55 ± 1.31 mg GAE/g) of *Ailanthus altissima*. Similarly, the TFC values from Luís et al. were lower in stems (8.04 ± 0.005 mg QE/g) and stalks (17.30 ± 0.63 mg QE/g), but the leaf extracts had a notably higher TFC of 87.09 ± 0.94 mg QE/g, comparable to our ethanolic extract.

Further, Cocîrlea et al. (2024) reported that the TPC of leaf extracts using a polar to non-polar sequential extraction method was 72.56 ± 0.14 mg GAE/g, with a TFC of 91.97 ± 0.53 mg QE/g. Using non-polar to polar sequential extraction, they obtained TPC values of 37.91 ± 0.04 mg GAE/g and TFC values of 60.20 ± 0.35 mg QE/g.

The differences in phenolic and flavonoid content across studies may be attributed to the varying extraction techniques, solvents, and plant parts used. Our results for water and ethanol extracts of *Ailanthus altissima* demonstrate a moderate phenolic content compared to the higher values found in other studies, particularly for leaves. The notably high flavonoid content in our ethanolic extracts is consistent with the high TFC values reported by Luís et al. and Cocîrlea et al., suggesting that ethanol may be particularly effective in extracting flavonoids from this species. Further exploration into the effect of extraction methods and plant part selection could provide a deeper understanding of the phytochemical composition of *Ailanthus altissima*.

The total phenolic content (TPC) for *Galinsoga parviflora* in our study was measured at 6.55 ± 1.15 mg GAE/g in water extracts and 11.75 ± 0.44 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was found to be 4.66 ± 0.03 mg QE/g in water extracts and 57.64 ± 1.20 mg QE/g in ethanol extracts.

Comparing these values to previous studies, Studzińska-Sroka et al. (2018) reported a significantly higher TPC of 98.30 ± 0.14 mg CA/g DW and a TFC of 6.15 ± 0.41 mg QE/g in *Galinsoga parviflora*. Similarly, Al-Robai et al. (2023) reported a TPC of 19.71 ± 0.51 mg GAE/g, with a TFC of approximately 10 mg QE/g.

The differences observed in our study, particularly the lower TPC values, may be attributed to differences in the plant parts used, solvent types, or geographic and environmental factors. The notably higher flavonoid content in our ethanol extracts (57.64 ± 1.20 mg QE/g) compared to the literature suggests that ethanol might be a more effective solvent for flavonoid extraction in *Galinsoga parviflora* under the conditions employed in this study. These findings highlight the variability in phytochemical content based on extraction methods and plant sources, warranting further research into optimizing extraction techniques for higher yields of bioactive compounds.

The total phenolic content (TPC) of *Acer negundo* was 18.77 ± 0.90 mg GAE/g in water extracts and 21.52 ± 1.54 mg GAE/g in ethanol extracts. The total flavonoid content (TFC) was 3.04 ± 0.03 mg QE/g in water extracts and 71.80 ± 2.67 mg QE/g in ethanol extracts. Since no comparative data for *Acer negundo* are available in the literature, these findings provide new insights into the phytochemical composition of this species. The higher flavonoid content in ethanol extracts suggests ethanol is particularly effective for flavonoid extraction. Further studies are needed to explore the influence of solvent selection and plant part on the phytochemical yield of *Acer negundo*.

The total phenolic content (TPC) of *Reynoutria japonica* was found to be 27.62 ± 0.98 mg GAE/g in water extracts and 16.69 ± 0.98 mg GAE/g in ethanol extracts, while the total flavonoid content (TFC) was 40.22 ± 1.64 mg QE/g in water extracts and 36.29 ± 0.78 mg QE/g in ethanol extracts.

Comparatively, Bozin et al. (2017) reported significantly higher TPC values for methanolic extracts from various plant parts, with the inflorescence showing 203.03 ± 6.07 mg GAE/g, and the rhizome with 186.45 ± 1.32 mg GAE/g. Stefanowicz et al. (2021) reported TPC values in tannic acid equivalents (TAE) for different plant parts, with leaves having 46 ± 6 mg TAE/g and stems 18 ± 6 mg TAE/g. The TFC reported by Bozin et al. for methanolic extracts was notably lower in leaves (2.84 ± 0.03 mg QE/g) and inflorescences (2.40 ± 0.08 mg QE/g) but showed higher values in the rhizome (16.31 ± 0.09 mg QE/g) and stems (18.21 ± 0.41 mg QE/g).

The differences between these findings and the current study can be attributed to variations in extraction solvents, plant parts, and environmental conditions. The notably higher flavonoid content observed in both water and ethanol extracts suggests that *Reynoutria japonica* is a rich source of flavonoids, especially in comparison to the values reported in methanolic extracts by other researchers.

No results were found in the literature for plant extracts from *Commelina communis*, *Panicum capillare*, and *Senecio inaequidens*, indicating a gap in research on these species. The findings presented in this study provide novel data on the phenolic and flavonoid content of these plants, contributing valuable insights to the current knowledge of their phytochemical composition.

CONCLUSION

The total phenolic content (TPC) and total flavonoid content (TFC) of the analyzed plant species demonstrate notable variation depending on the extraction solvent and plant part used. In most cases, ethanol extracts exhibited higher flavonoid content, while water extracts often yielded higher phenolic content, underscoring the importance of solvent selection in optimizing the extraction of specific compounds. Comparison with existing literature highlights substantial differences in TPC and TFC values, which can be attributed to variations in extraction methods, environmental factors, and plant material. The correlation between total flavonoid content (TFC) and total phenol content (TPC) is often influenced by various environmental factors such as temperature, light intensity, soil composition, and water availability. The environmental factors such as temperature, light intensity, soil composition, and water availability or nutrient deficiency may also trigger increased accumulation of these secondary metabolites. Therefore, TFC and TPC often show a positive correlation under environmental stress, suggesting a shared regulatory response to external stimuli.For certain species, such as *Acer negundo*, *Amaranthus albus*, *Commelina communis*, *Panicum capillare*, and *Senecio inaequidens*, limited or no data were available in the literature, making these findings an important contribution to the understanding of their phytochemical profiles. Ethanol was particularly effective in extracting flavonoids, especially in *Acer negundo* and *Reynoutria japonica*, while species like *Rhus typhina* and *Ailanthus altissima* showed strong phenolic and flavonoid presence across both solvents.

These results offer valuable insights into the potential bioactivity of these plants and lay the foundation for further research into their antioxidant and medicinal properties.

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Summary/Sažetak

Strane biljne vrste predstavljaju značajnu prijetnju prirodnim ekosistemima zbog svoje prilagodljivosti i invazivnog potencijala. U ovom radu ispitivana je antioksidativna aktivnost 13 stranih i invazivnih biljnih vrsta, s fokusom na ukupni sadržaj fenola, sadržaj flavonoida, ukupni antioksidativni kapacitet, ukupni oksidativni kapacitet te antimikrobnu aktivnost. Biljna tkiva su macerirana, isparena do suhoće, a ekstrakti su rastvoreni u vodi. Ukupni sadržaj fenola varirao je od 6,55 mg GAE/g ekstrakta do 30,82 mg GAE/g ekstrakta. Sadržaj flavonoida kretao se od 3,04 mg kvercetina/g ekstrakta do 40,81 mg kvercetina/g ekstrakta. Ukupni antioksidativni kapacitet iznosio je između 662 CEAC µM i 1540 CEAC µM. Ukupni oksidativni kapacitet može se povezati s abiotskim stresom. *Rhus typhina L.* i *Impatiens balfourii Hooker f.* pokazale su pozitivne vrijednosti ukupnog oksidativnog kapaciteta: 122,56 ± 7,85 µM H_2O_2 ekv./L i 141,38 ± 4,33 µM H_2O_2 ekv./L, redom. Ove dvije vrste su također pokazale najviši ukupni antioksidativni kapacitet, koji je iznosio 1190,06 ± 137,36 CEAC µM za *Rhus typhina L.* i 1540,34 ± 270,84 CEAC µM za *Impatiens balfourii* Hooker f. Rezultati sugeriraju da rastvarač za ekstrakciju značajno utiče na antioksidativni kapacitet, pri čemu etanol općenito omogućava veću efikasnost ekstrakcije kod mnogih vrsta. Antimikrobna aktivnost određena je protiv *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* i *Candida albicans*. *Rhus typhina L.* je pokazala aktivnost protiv *E. coli* i *P. mirabilis*. Ovi podaci ukazuju na to da biljke pojačavaju antioksidativne odbrambene sisteme kao odgovor na abiotske stresove. Jasna povezanost između vrijednosti ukupnog oksidativnog kapaciteta i ukupnog antioksidativnog kapaciteta kod biljaka predstavlja vrijedan pokazatelj mehanizma biljne odbrane.

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Content of Total Phenolics, Total Flavonoids and Vitamin C, and Antioxidant Activity of Selected Raspberry Varieties

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Abstract: This paper presents the study of total phenolics (TPs), total flavonoids (TFs), vitamin C and antioxidant activity (AA) of fruits and juices of raspberry varieties from the area of the municipality of Cazin. Five raspberry varieties (Fertodi, Meeker, Polka, Tulameen, and Willamette) grown according to the integral concept, as well as one sample of the Willamette variety grown as a hobby within the homestead - semi-intensive concept (W-sc) were analyzed. The TPs (before and after precipitation with formaldehyde) were quantified using the Folin-Ciocalteu method with gallic acid (GA) as a standard. The TFs were calculated from the difference between the TPs before and after precipitation. To determine the content of vitamin C, L-ascorbic acid (AsA) was utilized as a standard. In addition, the AA of the raspberry samples was tested using the ABTS radical scavenging method, wuth trolox (T) as a standard. The highest TPs content in fresh raspberry fruits (f.f.) was shown by the Tulameen variety (1608.77 mg GAE/100 g), while the W-sc sample showed the highest TPs content (1417.65 mg GAE/100 mL) among fresh raspberry juices (f.j.). The Tulameen variety had the highest TFs in f.f. (1513.81 mg GAE/100 g) and the Willamette variety in f.j. (789.99 mg GAE/100 mL). The highest content of vitamin C was shown by the variety Meeker in both tested raspberry fractions (45.87 mg AsAE/100 g in f.f., and 37.42 mg AsAE/100 mL in f.j.). All samples exhibited AA, whereby the best AA was shown by the Fertodi variety (7.60 mM TE/g) for f.f. samples, while the Meeker variety had the highest value of AA (6.26 mM TE/mL) among f.j. samples.

INTRODUCTION

Phenolic compounds present in plant species are an integral part of human nutrition and are of key interest due to their antioxidant (Cotelle *et al.*, 1996; Zheng and Wang, 2001), anti-inflammatory (Ferrándiz and Alcaraz, 1991; Wu *et al.*, 2006), anticancer (Brusselmans *et al.*, 2005; Angst *et al.*, 2013), antimicrobial and other beneficial effects (López-Lázaro, 2009; Kedika *et al.*, 2016). However, it should be emphasized that many of the phenolic compounds can have harmful effects at specific concentrations and conditions (Boots *et al.*, 2007; López-Lázaro, 2009).

Fruits, vegetables and numerous beverages are the main sources of phenolic compounds in the human diet. Significant amounts of by-products rich in phenols are produced by the food processing industry, which could later be valuable natural sources of antioxidants. Some of these by-products are the subject of research Their antioxidant activity was demonstrated through testing on edible oils, as well as fish and meat products, where the obtained extracts exhibited antioxidant properties comparable to various synthetic antioxidants (Balasundram *et al.*, 2006).

Extraction using organic solvents is the main method of phenols isolation, while the detection, identification and quantification of phenolic compounds use spectrophotometric (Folin-Ciocalteu test, Folin-Denis test, Prussian blue test, etc.), chromatographic (high-performance liquid chromatography), and more recently mass spectrometry methods (Khoddami *et al.*, 2013; Akimoto *et al.*, 2017).

The aim of this research is the determination of the content of total phenolics (TPs), total flavonoids (TFs) and vitamin C, as well as antioxidant activity (AA) in different raspberry varieties.

MATERIALS AND METHODS

Chemicals and reagents

Formaldehyde was obtained from Analitika, Sarajevo, Bosnia and Herzegovina. Sodium carbonate, hydrochloric acid and ethanol were obtained from Semikem, Sarajevo, Bosnia and Herzegovina. Potassium persulfate was purchased from Kemika, Zagreb, Croatia. Folin-Ciocalteu reagent and ammonium chloride were purchased from Carlo Erba Reagents, Milan, Italy. Gallic acid, L-ascorbic acid, ABTS and trolox were obtained from Sigma Aldrich, and *o*-phenylenediamine (OPD) was purchased from Merck, Darmstadt, Germany. Ammonium hydroxide was obtained from Panreac, Spain. All chemicals and reagents were analytical grade.

Equipment

UV/Vis spectrometer Lambda 25 (Perkin-Elmer) was used for determining the TPs and TFs contents, and for examining the AA in raspberry samples. The vitamin C content in these samples was determined using the luminescence spectrometer LS 55 (Perkin-Elmer). The samples were centrifuged on the microcentrifuge 22R Hettich. The analytical balance Mettler Toledo AB 104 was utilized for weighing the samples.

Methods

Determination of total phenolics

For the determination of TPs, a spectrophotometric method with Folin-Ciocalteu (F-C) reagent (Singleton and Rossi, 1965) was used, modified and adapted to experimental conditions by Keskin-Šašić *et al.* (2012).

The principle of this method is based on the oxidation of phenol with F-C reagent (yellow color) and the colorimetric measurement of the formed metal complexes (blue color). Under appropriate experimental conditions, the intensity of the blue coloration is directly proportional to the amount of phenol. An aqueous solution of gallic acid was used as a standard (Keskin-Šašić, 2013).

Preparation of standard solution of gallic acid

The amount of 0.0515 g of gallic acid (GA) was dissolved in 200 µL of 96% ethanol and transferred to a 100 mL measuring vessel after washing and refilling with distilled water.

This represented a standard GA solution with a mass concentration of 515 mg/L (stock solution). Working solutions (10.3; 30.9; 51.5; 72.1; 92.7; 113.3; 133.9 and 154.5 mg/L) were prepared from this stock solution to construct the calibration curve.

Recording the spectrum and drawing the calibration curve

After the preparation of GA working solutions of the specified concentrations, GA solutions with F-C reagent and sodium carbonate were prepared as follows:

- 1250 µL of F-C reagent (1:10) was added to 250 µL of GA working solution;
- after 10 minutes, 1000 µL of 7.5% Na_2CO_3 aqueous solution was added;
- with the prepared measurement solutions of GA (which amounted to 1.0; 3.1; 5.1; 7.2; 9.3; 11.3; 13.4 and 15.5 mg/L), absorbances were recorded at the maximum absorption, which was 743 nm.

The absorption maximum was determined by recording the absorption spectrum of a GA solution with a concentration of 5 mg/L, prepared as described above.

The relationship between absorbance and the concentration of GA measuring solutions was demonstrated, and the calibration curve equation was applied for further calculations of the TPs content in the samples. After performing the just-described procedures of appropriate preparation of the samples and recording of their absorbances, the results were converted to the TPs content expressed in GA equivalents by mass or fresh juice of sample [mg GAE/100 g(mL)].

Samples

Samples of five raspberry varieties („Fertodi“, „Meeker“, „Polka“, „Tulameen“ and „Willamette“) grown on plantations using the conventional concept, as well as one sample of the „Willamette“ variety grown as a hobby in the garden (semi-intensive concept) were collected in 2018 year from the area of the municipality of Cazin during the harvest period and were kept at a temperature of -32 °C until analysis.

Sample preparation and absorbance measurement

The amount of 1 g of a raspberry fruit sample was weighed and dissolved in 10 mL of distilled water. For the analysis of raspberry juice, 9 mL of distilled water was added to 1 mL of free-squeezed juice. 1250 µL of F-C reagent (1:10) was added to 250 µL of 10% raspberry homogenate. After 10 minutes 1000 µL of Na_2CO_3 solution was added to the sample. Absorbance was measured after 30 min. incubation at room temperature. All raspberry samples were analyzed in triplicate under the same experimental working conditions as with the GA standard.

Calculation of the content of total phenolics

The TPs content in the tested raspberry samples is expressed in mg GAE/100 g(mL) and can be calculated from the corresponding equation of the calibration curve ($y = 0.0087x - 0.0304$) and using a dilution factor ($f = 2.5$ mL/0.250 mL).

Determination of total flavonoids by the combined technique of spectrophotometric method and precipitation with formaldehyde

Total flavonoids were determined using the method of Ough and Amerine (1988). First, the absorbance was recorded for the tested sample, the value of which corresponds to total phenolics (flavonoid and non-flavonoid fractions). Then the flavonoid fraction was precipitated using formaldehyde (which reacts with flavonoids to form condensed products) and removed by centrifugation. Afterwards, the absorbance corresponding to the non-flavonoid fraction is measured in the remaining supernatant. The difference in the contents of phenolics obtained from these absorbances (before and after precipitation) corresponds to the flavonoid fraction. The obtained results are expressed as GAE per mass/volume of fresh fruit/juice of raspberry (mg GAE/100 $g_{f.f./f.j.}$).

Sample preparation and precipitation with formaldehyde

The sample, hydrochloric acid (HCl, 1:4) and formaldehyde (8 mg/L) were mixed in a ratio of 2:2:1. The solution thus prepared was mixed well and left to stand at room temperature for 24 hours, after which it was centrifuged at 8500 rpm for 10 minutes. The remaining phenolic compounds (non-flavonoid fraction) were determined in the supernatant according to the method for determining of TPs. The difference between the TPs content and the content of the non-flavonoid fraction gives the TFs values.

Determination of vitamin C

The spectrofluorimetric method for quantifying vitamin C/L-ascorbic acid (AsA) is based on its oxidation to L-dehydroascorbic acid, which reacts with *o*-phenylenediamine (OPD) in a basic medium to give an N-heterocyclic compound with a large number of conjugated π-bonds, which can emit strong fluorescence. The fluorescence intensity of the resulting compound is proportional to concentration and is measured at an excitation wavelength (λ_{ex}) of 360nm and an emission wavelength (λ_{em}) of 430 nm (Wu *et al.*, 2003).

The amount of 0.1068 g of AsA was weighed and dissolved in 100 mL of distilled water. This represented a standard AsA solution with a mass concentration of 1068 µg/mL (stock solution). Working solutions (1.07; 10.68; 32.04; 53.04; 74.76 and 96.12 µg/mL) were prepared from stock solution to construct the calibration curve.

Around 1 g of a raspberry fruit sample was weighed and macerated with 9 mL of distilled water, and then centrifuged at 15000 rpm for 15 minutes. A 1/10 diluted sample from the obtained supernatants was prepared. In the case of a raspberry juice sample, the sample was centrifuged under the same conditions and diluted in the same way as a fruit sample.

Solutions of AsA and samples prepared in this way were used for measurement. The solutions were mixed as follows: 1 mL AsA, 1 mL 0.5% OPD, and 1.5 mL buffer NH_4OH-NH_4Cl (pH = 9.4). After that, the solution was diluted to 10 mL with distilled water, mixed well and left to stand for 5 minutes before measuring. Fluorescence intensity was measured in a 1 cm quartz cuvette at an λ_{ex} of 360 nm and an λ_{em} of 430 nm.

Based on the calculated concentrations of AsA in the samples, results were expressed as mg AsA/100 g or mg AsA/100 mL of the sample.

Determination of antioxidant activity by ABTS method

The ability of the sample components to reduce the $ABTS^{\bullet+}$ radical cation (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was measured spectrophotometrically, where during the reaction the green $ABTS^{\bullet+}$ is reduced to its colorless form. Equal volumes of ABTS dissolved in water (7 mM) and $K_2S_2O_8$ (2.45 mM) were mixed and left for 12-16 hours in the dark at a temperature of 4 °C (Scalzo *et al.*, 2005). In this way, ABTS is converted into its $ABTS^{\bullet+}$. This solution is diluted that its absorbance is 0.7-0.9. After adding 100 µL of the sample to the diluted $ABTS^{\bullet+}$ solution, the absorbance is measured after 7 min. at 734 nm. Ethanol was used as a blank test, and trolox was used as a positive test.

The percentage of $ABTS^{\bullet+}$ radical cation inhibition is calculated according to the formula:

$$\mathrm{AA}\,(\%) = [(\mathrm{A_a} - \mathrm{A_b})/\mathrm{A_a}] \cdot 100$$

where is it:

- A_a - the absorbance of the ABTS solution before the addition of the sample (t = 0 min.);
- A_b - the absorbance of the solution after the addition of the sample (t = 7 min.).

The percentage of the antioxidant activity was measured as a function of the sample concentration, and the value of mM TE/g or mM TE/mL of the sample was calculated based on the equation of the calibration curve for trolox.

RESULTS AND DISCUSSION

Content of total phenolics in raspberry

The calibration curve method was used to determine TPs in raspberry samples, and it was constructed by measuring the absorbance of standard GA solutions of certain concentrations.

Values of TPs content in the samples of fresh raspberry fruits and juices are listed in Table 1:

Table 1. TPs content in the samples of fresh raspberry fruit and juice.

Raspberry	TPs ± SD (mg GAE/100 g)	TPs ± SD (mg GAE/100mL)
„Tulameen“	**1608.77 ± 72.71**	1003.30 ± 156.98
„Willamette“	1604.81 ± 33.01	998.40 ± 72.07
„Fertodi“	1525.01 ± 65.65	423.27 ± 38.36
W-sc*	1504.09 ± 26.97	**1417.65 ± 132.76**
„Meeker“	1457.06 ± 56.27	1006.45 ± 166.02
„Polka“	1443.82 ± 36.50	154.66 ± 23.10

*a sample of the „Willamette“ variety grown as a hobby within the home garden - a semi-intensive concept.

Content of total flavonoids in raspberry

Values of TFs content in the samples of fresh raspberry fruits and juices are listed in Table 2:

Table 2. TFs content in the samples of fresh raspberry fruit and juice.

Raspberry	TFs ± SD (mg GAE/100 g)	TFs ± SD (mg GAE/100mL)
„Tulameen“	**1513.81 ± 6.86**	636.49 ± 43.08
„Willamette“	1488.40 ± 1.50	**789.99 ± 27.33**
W-sc*	1398.75 ± 7.35	618.80 ± 66.24
„Fertodi“	1386.01 ± 5.28	184.79 ± 6.21
„Meeker“	1353.44 ± 1.89	540.53 ± 6.34
„Polka“	1345.58 ± 1.62	100.43 ± 0.03

*a sample of the „Willamette“ variety grown as a hobby within the home garden - a semi-intensive concept.

Content of vitamin C in raspberry

The calibration curve method was used to determine vitamin C in raspberry samples, and it was constructed by measuring of the relative intensity of fluorescence of standard AsA solutions of certain concentrations.

Vitamin C content in the samples of fresh raspberry fruits and juices is listed in Table 3:

Table 3. Vitamin C content in the samples of fresh raspberry fruits and juices.

Raspberry	Vitamin C (mg/100 g)	Vitamin C (mg/100 mL)
„Meeker“	**45.87**	**37.42**
„Tulameen“	43.96	20.34
„Willamette“	36.27	3.17
W-sc*	32.27	15.76
„Polka“	29.03	5.29
„Fertodi“	28.87	29.68

*a sample of the „Willamette“ variety grown as a hobby within the home garden - a semi-intensive concept.

Antioxidant activity in raspberry samples

The ABTS method was used to determine the antioxidant activity (AA) in raspberry samples.

Values of AA of fresh raspberry fruits and juices are listed in Table 4:

Table 4. Antioxidant activity in the samples of fresh raspberry fruits and juices.

Raspberry	AA ± SD (mM TE/g)	AA ± SD (mM TE/mL)
„Fertodi“	**7.60 ± 4.60**	4.81 ± 0.03
„Meeker“	7.48 ± 2.83	**6.26 ± 0.02**
W-sc*	6.71 ± 1.98	5.20 ± 0.21
„Polka“	4.06 ± 2.79	1.33 ± 0.08
„Tulameen“	3.81 ± 1.44	4.80 ± 0.03
„Willamette“	2.56 ± 0.90	5.07 ± 0.18

*a sample of the „Willamette“ variety grown as a hobby within the home garden - a semi-intensive concept.

Five raspberry varieties grown with the integral concept were analyzed: „Fertodi“, „Meeker“, „Polka“, „Tulameen“, and „Willamette“, as well as one sample of the „Willamette“ variety grown as a hobby within the homestead semi-intensive concept (W-sc). Student's t-test was used for statistical analysis.

The content of TPs in the fresh fruit of the examined raspberries was in the interval from 1443.82±36.50 to 1608.77±72.71 mg GAE/100 g, with the highest average content found in the variety „Tulameen“ (1608.77±72.71), followed by „Willamette“ (1604.81±33.01), „Fertodi“ (1525.01±65.65), raspberry sample W-sc (1504.09±26.97), „Meeker“ (1457.06±56.27), while the lowest TPs content was measured in „Polka“ fruit (1443.82±36.50) (Table 1).

There is a statistically significant difference in the TPs content in the fresh fruit of the variety „Tulameen“ compared to the TPs content in the fresh fruit of the varieties „Meeker“ and „Polka“ ($p^{*}<0.05$; Student's t-test), as well as compared to the TPs content of the fruit W-sc ($p<0.05$). Then, there is a statistically significant difference in the TPs content in the fresh fruit sample of the „Willamette“ variety compared to the TPs content in the fresh fruit of the „Meeker“ and „Polka“ varieties, as well as compared to the TPs content in the fresh fruit of the raspberry W-sc sample ($p^{**}<0.01$). Also, there is a statistically significant difference in the TPs content in the fresh fruit of the W-sc raspberry sample versus the TPs content in the fresh fruit of the variety „Polka“ ($p^{*}<0.05$).

The TPs content in the fresh juice of the tested raspberries ranged from 154.66±23.10 to 1417.65±132.76 mg GAE/100 mL, with the highest average content found in the W-sc raspberry sample (1417.65±132.76), followed by „Meeker“ (1006.45±166.02), „Tulameen“ (1003.30±156.98), „Willamette“ (998.40±72.07), „Fertodi“ (423.27±38.36), while the lowest TPs content was found in the variety „Polka“ (154.66±23.10) (Table 1). A statistically significant difference was in the TPs content in the fresh juice of the W-sc raspberry sample compared to the TPs content in all the fresh juices of the five raspberry varieties grown with the integral concept on the plantations, $p^{***}<0.001$ compared to the varieties „Fertodi“ and „Polka“, $p^{**}<0.01$ compared to the variety „Willamette“, $p^{*}<0.05$ compared to the varieties „Meeker“ and „Tulameen“.

The content of TFs in the fresh fruit of the tested raspberries ranged from 1345.58±1.62 to 1513.81±6.86 mg GAE/100 g, with the highest average content obtained for the variety „Tulameen“ (1513.81±6.86), followed by „Willamette“ (1488.40±1.50), raspberry sample W-sc (1398.75±7.35), „Fertodi“ (1386.01±5.28), „Meeker“ (1353.44±1.89), while the lowest average TFs content was found in the fruit of the variety „Polka“ (1345.58±1.62) (Table 2).

The content of TFs in the fresh fruit of the variety „Tulameen“ was statistically significantly higher compared to TFs content in all analyzed raspberries, $p^{***}<0.001$ compared to the varieties „Fertodi“, „Meeker“, „Polka“, raspberry sample W-sc, and $p^{**}<0.01$ compared to „Willamette“. Also, the content of TFs in the fresh fruit of the variety „Willamette“ was statistically significantly higher compared to the content of TFs in raspberry varieties „Fertodi“, „Meeker“, „Polka“ and raspberry sample W-sc ($p^{***}<0.001$).

Šapčanin *et al.* (2017) have determined the content of TFs in ethanolic extract of the raspberry. The spectrophotometric method based on forming a flavonoid complex with aluminum and quercetin (Qu) as the standard were used in this study. They found that the content of TFs was 1.02 mg QuE/g of fresh weight of the sample.

Çekiç and Özgen (2010) have examined the content of TPs in raspberry varieties „Heritage“ and „Tulameen“. Obtained values for these varieties were 1795 and 2342 µg GAE/g_{fw}, respectively.

Furthermore, the content of TPs and TFs of the „Willamette“ red raspberry (southwestern Serbia) under conventional farming was estimated by Murtić *et al.* (2023). The results for the previously mentioned raspberry variety from 2021 were 19.11±1.51 mg GAE/g (TPs) and 7.14±0.24 mg CE/g (TFs), and from 2022 were 19.71±1.73 mg GAE/g and 7.18±0.43 mg CE/g.

The content of TFs in fresh raspberry juice ranged in the interval 100.43±0.03-789.99±27.33 mg GAE/100 mL, with the highest average content obtained for the variety „Willamette“ (789.99±27.33), followed by „Tulameen“ (636.49±43.08), sample W-sc (618.80±66.24), „Meeker“ (540.53±6.34), „Fertodi“ (184.79±6.21), while the lowest TFs content was found in the juice of the variety „Polka“ (100.43±0.03) (Table 2).

It is observed that raspberry fruit is significantly richer in TPs and TFs content than raspberry juice. Statistical analysis showed that the content of TPs in the fruit of the analyzed raspberries is statistically significantly higher compared to the content of TPs in the juice of the same raspberries ($p^{**}<0.01$), and the same applies when it comes to the content of TFs, with the fact that this difference is still more pronounced ($p^{***}<0.001$).

According to most of the available literature data, the levels of TPs in fresh fruits (fw) of raspberries are in the interval 428-1079 mg/100 g_{fw} for black fruit, 192-512 mg/100 g_{fw} for red fruit, 428-451 mg/100 g_{fw} for pink-red fruit and 241-359 mg/100 g_{fw} for yellow fruit. Anthocyanins are the main phenolics in black raspberries, with levels in the interval 464-627 mg/100 g_{fw} (Zhao, 2007).

Based on the obtained results and comparison with literature data, it can be concluded that raspberries from the area of Cazin municipality are extremely rich in the content of TPs and TFs. Also, for a more complete intake of polyphenols, it is preferable to consume the whole raspberry fruit, in comparison to its juice.

The content of vitamin C in the fresh fruit of the tested raspberries ranged from 28.87 to 45.87 mg/100 g, with the highest content obtained for the variety „Meeker“ (45.87), followed by „Tulameen“ (43.96), „Willamette“ (36.27), W-sc raspberry sample (32.27), „Polka“ (29.03), while the lowest vitamin C content found in the fruit of the variety „Fertodi“ (28.87) (Table 3).

On the other hand, the content of vitamin C in the fresh raspberry juice ranged in the interval 3.17-37.42 mg/100 mL, with the highest content obtained for the variety „Meeker“ (37.42), followed by „Fertodi“ (29.68), „Tulameen“ (20.34), sample W-sc (15.76), „Polka“ (5.29), while the lowest content of vitamin C was found in the juice of the variety „Willamette“ (3.17) (Table 3).

Vitamin C content in the fruit of six different raspberry varieties („Willamette“, „Meeker“, „Malahat“, „Tulameen“, „Chilliwack“, „Glen Ample“) has been estimated by Milivojević *et al.* (2012). Toward their study, the highest vitamin C content was found in the variety „Chilliwack“ (52.3 mg/100 g_{fw}), whereas the „Tulameen“ variety showed the lowest vitamin C content (38.4 mg/100 g_{fw}).

Ponder and Hallmann (2020) have determined the content of vitamin C in the fruit of some raspberry varieties. They have reported that the highest content of vitamin C was found in the „Tulameen“ variety (42.5±2.8 mg/100 g_{fw}), which was collected in 2013 at summer time. However, among the raspberry samples collected in 2014 at summer time, the variety „Glen Fine“ showed the highest content of vitamin C (42.7±4.7 mg/100 g_{fw}).

The values of AA in the fruit of the raspberry samples ranged in the interval 2.56-7.60 mM TE/g. The variety „Fertodi“ showed the best AA (7.60±4.60), while the lowest value of AA was measured in the fruit of the variety „Willamette“ (2.56±0.90) (Table 4).

In the juice of the raspberry samples, the values of AA ranged from 1.33 to 6.26 mM TE/mL. The best AA was shown by the variety „Meeker“ (6.26±0.02), while the variety „Polka“ showed the lowest AA (1.33±0.08) (Table 4).

Sariburun *et al.* (2010) used the ABTS method to estimate the AA in the fruit of certain raspberry varieties, which were collected in the region of Kestel (Bursa, Turkey). The best AA was shown by the methanol extract of the fruit of the variety „Hollanda Boduru“ (117.07±0.94 µmol TE/g_{fw}).

CONCLUSIONS

In this study, the content of certain bioactive compounds (total phenolics, total flavonoids, vitamin C), as well as the antioxidant activity of the selected raspberry varieties were estimated. The obtained results showed that the tested raspberry varieties from the area of Cazin municipality are extremely rich in total phenolics and total flavonoids content. Statistical analysis showed that the fruit of the analyzed raspberries possesses a statistically significantly higher content of total phenolics and total flavonoids compared to their juice. Therefore, it is more advisable to consume the whole raspberry fruit. Based on the comparison with the literature data, it can be concluded that raspberry fruits are a very good source of vitamin C. Also, the raspberry samples showed significant antioxidant activity, which correlates with the presence of previously determined bioactive compounds.

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Summary/Sažetak

U ovom radu prikazano je istraživanje ukupnih fenola (UF), ukupnih flavonoida (UFL), vitamina C i antioksidativne aktivnosti (AA) plodova i sokova sorti maline sa područja općine Cazin. Analizirano je pet sorti maline (Fertodi, Meeker, Polka, Tulameen i Willamette) uzgajanih integralnim konceptom, kao i jedan uzorak sorte Willamette uzgajan iz hobija unutar okućnice - poluintenzivni koncept (W-pk). UF (prije i poslije taloženja formaldehidom) kvantificirani su Folin-Ciocalteu metodom sa galnom kiselinom (GA) kao standardom. UFL izračunati su iz razlike između UF prije i poslije taloženja. Za određivanje sadržaja vitamina C korištena je L-askorbinska kiselina (AsA) kao standard. Osim toga, AA uzoraka maline ispitana je metodom hvatanja ABTS radikala, koristeći troloks (T) kao standard. Najviši sadržaj UF u svježim plodovima (s.p.) maline pokazala je sorta Tulameen (1608,77 mg GAE/100 g), dok je uzorak W-pk pokazao najviši sadržaj UF (1417,65 mg GAE/100 ml) među svježim sokovima (s.s.) maline. Sorta Tulameen imala je najviše UFL u s.p. (1513,81 mg GAE/100 g), a sorta Willamette u s.s. (789,99 mg GAE/100 ml). Najviši sadržaj vitamina C pokazala je sorta Meeker u obje ispitivane frakcije maline (45,87 mg AsAE/100 g u s.p., odnosno 37,42 mg AsAE/100 ml u s.s.). Svi uzorci pokazali su AA, pri čemu je najbolju AA pokazala sorta Fertodi (7,60 mM TE/g) za uzorke s.p., dok je među uzorcima s.s. sorta Meeker imala najvišu vrijednost AA (6,26 mM TE/ml).

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ODLUKU O RAZRJEŠENJU PREDSJEDNIKA, POTPREDSJEDNIKA I GLAVNOG UREDNIKA DRUŠTVENIH GLASILA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti predsjednik, potpredsjednik i glavni urednik društvenih glasila Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Dragan Krešić, predsjednik
2. Šaćira Mandal, potpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-1/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O RAZRJEŠENJU PREDSJEDNIKA I ČLANOVA Upravnog Odbora Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Upravnog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Jelena Ostojić, predsjednik
2. Selma Burović, potpredsjednik
3. Selma Korać, sekretar
4. Sabina Begić, blagajnik
5. Safija Herenda, urednik glasila
6. Dinaida Tahirović, član
7. Anes Krečo, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-2/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O RAZRJEŠENJU ČLANOVA
Nadzornog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Nadzornog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ :

1. Nevzeta Ljubijankić, predsjednik
2. Amira Čopra Janićijević, član
3. Lejla Klepo, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-3/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O RAZRJEŠENJU ČLANOVA
Suda časti
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti članovi Suda časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Danijela Vidic, član
2. Ismet Tahirović, član
3. Sabina Gojak-Salimović, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-4/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O IMENOVANJU ČLANOVA Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Skupštinu udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Safija Herenda, predsjednik
2. Dragan Krešić, potpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

Član 2.

Izabrani članovi Skupštine Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Skupštine traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-01/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O IMENOVANJU PREDSJEDNIKA I ČLANOVA Upravnog Odbora Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Upravni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Sabina Begić, predsjednik
2. Jelena Ostojić, potpredsjednik
3. Irnesa Osmanković, sekretar
4. Lejla Klepo, blagajnik
5. Anes Krečo, član ispred glasila
6. Dinaida Tahirović, član
7. Emina Opanković, član

Član 2.

Izabrani članovi Upravnog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Upravnog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-02/25
03.04.2025. godine
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ODLUKU O IMENOVANJU ČLANOVA Nadzornog Odbora Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Nadzorni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Mirel Subašić, predsjednik
2. Mirha Pazalja, član
3. Selma Burović, član

Član 2.

Izabrani članovi Nadzornog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Nadzornog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-03/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O IMENOVANJU ČLANOVA
Suda časti
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Sud časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Danijela Vidic, član
2. Šaćira Mandal, član
3. Sabina Gojak-Salimović, član

Član 2.

Izabrani članovi Suda časti su postali kandidati sa najvećim brojem glasova. Mandat članova Suda časti traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-04/25
03.4.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O RAZRJEŠENJU I IMENOVANJU PREDSJEDNIKA Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješava se dužnosti predsjednik udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

Jelena Ostojić, predsjednik Društva

Član 2.

Imenuje se predsjednik udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

Sabina Begić, predsjednik Društva

Član 3.

Izabrani predsjednik Udruženja je izabran sa najvećim brojem glasova. Mandat predsjenika udruženja traje 2 godine.

Broj: P-02-05/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O RAZRJEŠENJU I IMENOVANJU PREDSJEDNIKA I POTPREDSJEDNIKA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

Razrješavaju se dužnosti predsjednik i potpredsjednik Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Dragan Krešić, predsjednik
2. Šaćira Mandal, potpredsjednik

Član 2.

Imenuju se predsjednik i potpredsjednik Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

3. Safija Herenda, predsjednik
4. Dragan Krešić, potpredsjednik

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-06/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU

o razrješenju i imenovanju lica za zastupanje i predstavljanje

Član 1.

Razrješavaju se dužnosti lica za zastupanje i predstavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Jelena Ostojić, predsjednik Društva
2. Selma Korać, član Upravnog odbora
3. Sabina Begić, član Upravnog odbora

Član 2.

Imenuju se lica za zastupanje i prestavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Sabina Begić, predsjednik Društva
2. Irnesa Osmanković, član Upravnog odbora
3. Lejla Klepo, član Upravnog odbora

Član 3.

Izabrana lica za zastupanje i predstavljanje su po članu 7. Statuta Udruženja DKTKS predsjednik Društva i dva odabrana člana Upravnog odbora. Mandat lica za zastupanje i predstavljanje traje 2 godine.

Član 4.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-03/25
03.04.2025. godine
Sarajevo

Predsjednik Skupštine

Safija Herenda

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ODLUKU O SPONZORSTVU „14. OTVORENIH DANA HEMIJE“

Član 1.

Upravni odbor je Društva na elektronskoj sjednici održanoj 9. 4. 2025. godine donio je odluku da se doniraju novčana sredstva u svrhu organizacije manifestacije “14. Otvoreni dani hemije” u iznosu od 450 KM (račun za promotivne čokoladice).

Broj: P-04/25
9. april 2025. godine
Sarajevo

Predsjednik Društva

Jelena Ostojić

INSTRUCTIONS FOR AUTHORS

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e) Patents:
Healey, P.J., Wright, S.M., Viltro, L.J., (2004).*Method and apparatus for the selection of oral care chemistry*, The Procter & Gamble Company Intellectual Property Division, (No.US 2004/0018475 A1).

f) Chemical Abstracts:
Habeger, C. F., Linhart, R. V., Adair, J. H. (1995). Adhesion to model surfaces in a flow through system. *Chemical Abstracts,* CA 124:25135.

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ISO 4790:1992. (2008). *Glass-to-glass sealings - Determination of stresses*.

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   mp 180°C dec.
   bp 98°C
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3. ***NMR Spectroscopy***:
   ^{1}H NMR (500 MHz, DMSO-d_6) d 0.85 (s, 3H, CH_3), 1.28–1.65 (m, 8H, 4´CH_2), 4.36–4.55 (m, 2H, H-1 and H-2), 7.41 (d, *J* 8.2 Hz, 1H, ArH), 7.76 (dd, *J* 6.0, 8.2 Hz, 1H, H-1'), 8.09 (br s, 1H, NH).
   ^{13}C NMR (125 MHz, $CDCl_3$) d 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).
   Abbreviations: d, chemical shift in parts per million (ppm) downfield from the standard; *J*, coupling constant in hertz; multiplicities s, singlet; d, doublet; t, triplet; q, quartet; and br, broadened. Detailed peak assignments should not be made unless these are supported by definitive experiments such as isotopic labelling, DEPT, or two-dimensional NMR experiments.
4. ***IR Spectroscopy***:
   IR (KBr) n 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm^{-1}.
   Abbreviation: n, wavenumber of maximum absorption peaks in reciprocal centimetres.
5. ***Mass Spectrometry***:
   MS *m/z* (relative intensity): 305 (M^+H, 100), 128 (25).
   HRMS–FAB (*m/z*): $[M+H]^+$calcd for $C_{21}H_{38}N_4O_6$, 442.2791; found, 442.2782.
   Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M^+, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.
6. ***UV–Visible Spectroscopy***:
   UV (CH_3OH) l_{max} (log e) 220 (3.10), 425 nm (3.26).
   Abbreviations: l_{max}, wavelength of maximum absorption in nanometres; e, extinction coefficient.
7. ***Quantitative analysis***:
   Anal.calcd for $C_{17}H_{24}N_2O_3$: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16.All values are given in percentages.
8. ***Enzymes and catalytic proteins relevant data:***
   Papers reporting enzymes and catalytic proteins relevant data should include the identity of the enzymes/proteins, preparation and criteria of purity, assay conditions, methodology, activity, and any other information relevant to judging the reproducibility of the results[1]. For more details check Beilstein Institut/STRENDA (standards for reporting enzymology data) commission Web site (http://www.strenda.org/documents.html).

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