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Biological potential and physicochemical characteristics of three plants from the Rhodopes, Bulgaria

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Abstract

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This study aims to investigate the biological potential and physicochemical characteristics of three plants from the Rhodope region, Bulgaria: blackberry (*Rubus fruticosus*) and aronia (*Aronia melanocarpa*) berries, and mint leaves (*Mentha arvensis*), which are known as aromatic and medicinal plants. The following physicochemical parameters were determined: moisture, ash, and dry matter. The berries contained a comparable amount of vitamin C (1551 mg/kg), which was about twice as high as the mint leaves (846 mg/kg). Aronia berries had the highest total carbohydrate content (18.26%), which was about twice as high as blackberry and mint leaves. The blackberry had the highest protein content (10.20%), followed by mint leaves (8.95%), and the aronia berries had the lowest content (6.46%). The content of total phenolics (TPC) and flavonoids (TFC) of methanolic extracts of mint leaves and both berries was measured. The highest TPC and TFC values were found in the mint leaf extract, 6.86 mg GAE/g dw and 2.17 mg GAE/g dw, respectively. High anthocyanin content was found in aronia and blackberry extracts with values of 180.52 mg cyd-3-glu/g dw and 190.45 mg cyd-3-glu/g dw, respectively. The antioxidant activity was also measured by two methods – radical-scavenging ability (DPPH) and ferric-reducing antioxidant power assay (FRAP). The results showed a high correlation between the DPPH and FRAP assays with TPC, with $r^2 = 0.7793$ and $r^2 = 0.9894$, respectively. Consequently, the TPC contributed to the antioxidant properties of the tested samples. The antimicrobial activity of the investigated methanolic extracts was determined. The results revealed the potential of the studied plant species for their application in pharmaceutical and functional food products.

Keywords: aronia and blackberry; mint leaves; total phenol and flavonoid content; DPPH and ABTS assay

1. Introduction

In recent decades, there has been an increased interest in identifying beneficial bioactive compounds of plant origin that can be added to foods and beverages to achieve beneficial health effects (Ye et al., 2021). A popular research topic is the use of essential oils (EOs) and fragrances in the food, pharmaceutical, and cosmetic industries, where they are widely used in various applications. They possess antioxidant and antimicrobial activity and can be used directly as ingredients or as a bioactive component. Plant antioxidants are mainly phenolic compounds, carotenoids, and vitamins (De-Montijo-Prieto et al., 2021; Abeyrathne et al., 2022).

The current scientific team has turned its attention to studying the biological potential and physicochemical characteristics of three plants from the region of Dospat, Bulgaria. The blackberry (*Rubus fruticosus*) and aronia (*Aronia melanocarpa*) berries, which contain functional and nutritional ingredients are characterized by high antioxidant potential. Mint leaves (*Mentha arvensis*) are recognized as an aromatic and medicinal plant exhibiting good antiviral properties.

1.1 Botanical and physicochemical characteristics of the plants

The blackberry (*Rubus fruticosus* L.) is a genus of shrubs from the rose family (*Rosaceae*). Almost 700 species represent *Rubus,* and it is the largest genus in this family. According to long-term research by the US Department of Agriculture (USDA), blackberries occupy the second place on the scale – "Oxygen Radical Absorbent Capacity" – 340 (ORAC value of 1 g of fruit) (Delkov, 1984; Memete et al., 2023). Memete et al. (2023) studied the fruit of blackberry (*Rubus* spp.) and found significant results. They found that it has a powerful antioxidant capacity due to the high levels of anthocyanins and other phenolics it contains. In terms of polyphenol content and antioxidant capacity, Memete et al. (2023) found that the major anthocyanin in blackberry was cyanidin-3-glucoside, with the highest amount recorded in the cultivar "No spikes" 329.26 ± 9.36 mg/g dw. The plant *Rubus* yields triterpenic acid and rubitonic acid, which are characterized as 7α-hydroxyursolic acid (Verlag, 2004). According to Condé (2013), blackberries are distinguished by their high content of dietary fiber, vitamin C, vitamin K, and the mineral manganese. Extracts from fruits that synthesize anthocyanins can be effective against harmful bacteria without harming the intestinal microbiota (Raudsepp et al., 2013).

Aronia (*Aronia melanocarpa*) is a deciduous shrub in the genus *Aronia,* family *Rosaceae*. It is believed to be native to North America and Canada. It was brought to Europe in the 18th century. The composition of aronia berries depends on many factors: variety, maturity, and climatic conditions. It was found by Mayer-Miebach et al. (2012) that the dry weight of the fruits was 11.1–17.4%. It was shown by Ochmian et al. (2012) that aronia berries contain 15.3–19.5% dry matter, including 14.2–18.7% soluble matter. The amount of protein is low, at 3.7 g/100 g DM (dry matter) (Cervenka et al., 2011). Different authors have found different content of polyphenols in aronia fruits, ranging from 778 to 7849 mg/100 g DM (Oszmianski and Wojdylo, 2005; Hudec et al., 2006; Teleszko and Wojdyło, 2015); 1079–2996 mg gallic acid equivalents/100 g fresh weight (Wangensteen et al., 2014); 819–1330 mg GAE/100 g FW (fresh weight) (Kulling and Rawel, 2008); 1285 g GAE/kg FW (Jing et al., 2008). Aronia products and waste are also rich in polyphenols (Tolic et al., 2015; Cujic, 2016). The total weight of the amino acid composition amounts to 28.9 g/kg DM (Parada and Aguilera, 2007; Lemmens et al., 2010). The total lipid content of fresh aronia berries is 0.09–0.17% (Merisko-Liversidge et al., 2003; Ross et al., 2011). DPPH analysis by Sidor and Gramza-Michałowska (2019) on the phenolic compounds and antioxidant potential of blackberry, blackcurrant, aronia, and raspberry fruits showed a relatively high potential of aronia (Benvenuti et al., 2004). Oziembłowski et al. (2022) studied the content of polyphenols in aronia affecting the extremely high antioxidant capacity of the fruit (Tarko et al., 2009; Trenka et al., 2020). After the research carried out by Valcheva-Kuzmanova and Belcheva (2006), it was found that aronia has anti-inflammatory, gastroprotective, antidiabetic, and hepatoprotective properties. Aronia fruit juice also has bacteriostatic activity *in vitro* against *Staphylococcus aureus* and *Escherichia coli* and antiviral activity against type A influenza virus.

Mint (*Mentha arvensis* L.) is a perennial herbaceous plant of the *Lamiaceae* family. Between 13 and 24 mint species are thought to exist, about 9 of which—with numerous variations—are found in Bulgaria. The pharmacological properties of the mint can be related to the presence of bioactive components such as terpenoids, alcohols, rosmarinic acid, and phenols (Anwar et al., 2019). A review performed by Tafrihi et al. (2021) highlighted the antimicrobial activity of compounds and essential oils derived from peppermint. Anwar et al. (2017) investigated the *in vitro* antioxidant activity of mint by two methods (DPPH and ABTS+). The authors stated that antimicrobial activity of mint is mainly due to volatile bioactive substances such as oxidized monoterpenoids, monoterpene hydrocarbons (MHs), and sesquiterpene hydrocarbons. Mint EOs have been found to exhibit antibacterial activity against pathogenic bacteria, including Gram-negative and Gram-positive, such as *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens* (Rice-Evans et al., 1997; Anwar et al., 2017; Saba

and Anwar, 2018). According to another study by Irshad et al. (2011), peppermint shows strong antibacterial effects, especially against Gram-negative strains, including *P. aeruginosa, E. coli,* and *S. enterica.* Orhan et al. (2012) reported that the essential oils of mint plants, *Mentha spicata* and *Mentha piperita*, contain compounds with antiviral activity, a finding also investigated by McKay and Blumberg (2006). In folk medicine, peppermint is used to treat upper respiratory tract conditions and acts as an antiseptic and analgesic for stomachaches and chest pains (Celenk et al., 2008; Venkatachalam et al., 2020; Haddou et al., 2023).

The present study aims to investigate the biological potential and physicochemical characteristics of three plants from the Rhodope Mountains region: blackberry (*Rubus fruticosus*) and aronia (*Aronia melanocarpa*) berries, and mint leaves (*Menth arvensis*), all known as aromatic and medicinal plants.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

In the present study, three plants from the Rhodope Mountains region, Bulgaria were analyzed: blackberry (*Rubus fruticosus*) and aronia (*Aronia melanocarpa*) berries, and mint (*Mentha arvensis*) leaves. The fruits and leaves were collected from July to September 2023, in the area of Dospat (Lyubcha village), Smolyan district (41°37′N 24°09′E), located in the Western Rhodopes, Bulgaria. Plant material was identified according to Herbarium Academiae Scientiarum Bulgariae. The plants were air-dried at temperature of 20-23°C, as shown in Table 1.

2.1.2 Test microorganisms

Twenty-two microorganisms including seven Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus amyloliquefaciens* 4BCL-YT, *Bacillus cereus* NCTC 11145, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* NBIMCC 8632, *Enterococcus faecalis* ATCC 19433 and *Micrococcus luteus* 2YC-YT), seven Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* NBIMCC 1672, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Proteus mirabilis* 56/10 and *Pseudomonas aeruginosa* ATCC 9027), two yeasts (*Candida albicans* NBIMCC 74 and *Saccharomyces cerevisiae* ATCC 9763) and six fungi (*Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Penicillium chrysogenum, Fusarium moniliforme* ATCC 38932, *Rhizopus* sp. *and Mucor* sp.) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria were selected for the antimicrobial activity test.

B. subtilis, B. amyloliquefaciens, B. cereus, and *M. luteus* were cultured on LBG agar at 30°C for 24h. In contrast, *S. aureus, L. monocytogenes, E. faecalis, E. faecium, S. enteritidis, S. typhimurium, K. pneumoniae, E. coli, P. vulgaris, P. mirabilis, and P. aeruginosa* were cultured on LBG agar at 37°C for 24 h. The yeast *C. albicans* was cultured on MEA at 37°C, while *S. cerevisiae* was cultured on MEA at 30°C for 24 h. The fungi *A. niger, A. flavus, P. chrysogenum, F. moniliforme, Rhizopus* sp., and *Mucor* sp. were grown on MEA at 30°C for 7 days or until sporulation.

2.1.3 Culture media

Luria-Bertani agar medium with glucose (LBG agar)

LBG agar was used for the cultivation of test bacteria. A quantity of 50 g of LBG-solid substance mixture (containing 10 g tryptone, 5 g yeast extract, 10 g NaCl, 10 g glucose, and 15 g agar) was dissolved in 1 L of deionized water, pH 7.5 ± 0.2.

Malt extract agar (MEA)

MEA was used for the cultivation of test yeasts and fungi. A quantity of 50 g of the MEA-solid substance mixture (containing 30 g malt extract, 5 g mycological peptone, and 15 g agar) was dissolved in 1 L of deionized water, pH 5.4 ± 0.2.

The culture media were prepared according to the manufacturer's instructions (Scharlab SL, Spain) and autoclaved at 121°C for 20 min before use.

2.2 Methods

2.2.1 Extracts preparation

The frozen and dried fruits were preliminarily ground using a blender. Four grams of each ground sample were weighed in a plastic tube, and then macerated with 40 ml of methanol (Sigma-Aldrich, Merck, Germany). The samples were stirred by vortex (V-1, Biosan, Latvia) for 10-15 s, and then left at room temperature for 48 h, in darkness. The obtained extracts were filtered through filter paper, and

Table 1. Origin of the three Bulgarian medicinal plants

Plant	Region	District	GPS Coordinates	Altitude, m
Blackberry *(Rubus fruticosus)*	Dospat Municipality, Lyubcha village	Smolyan	41°38′N 24°06′E	1190
Aronia *(Aronia melanocarpa)*	Dospat Municipality, Lyubcha village	Smolyan	41°61′N 24°05′E	1175
Mint *(Mentha arvensis)*	Dospat Municipality, Lyubcha village	Smolyan	41°61′N 24°09′E	1170

Source: Authors' own elaboration

then stored at 4°C until analyses. To determine the anti-inflammatory activity, the methanol was vacuum evaporated, and the extracts were diluted in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Merck, Germany).

2.2.2 Physicochemical analysis

Physicochemical characteristics of the three plants were assessed according to the following Bulgarian State Standards: moisture content – BSS ISO 939:2021, ash content – BSS ISO 928:2004, carbohydrates – BSS 7169:1989, proteins – BSS 15438:1982, and ascorbic acid (vitamin C) – BSS 11812:1991.

2.2.3 Total phenolic content

The total phenolic content (TPC) was determined using a Folin-Ciocalteu reagent. The reaction mixture containing 1 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, Merck, Germany), 0.8 ml of 7.5% sodium carbonate (Sigma-Aldrich, Merck), and 0.2 ml of the plant extract was kept at room temperature for 20 min, in darkness. The absorbance was measured by a spectrophotometer Camspec M107 (Spectronic-Camspec Ltd., UK) at 765 nm against a blank (distilled water). The results were presented as mg equivalent of gallic acid (GAE)/g of dry weight (dw) sample (Ivanov et al., 2014).

2.2.4 Total flavonoid content

The total flavonoid content (TFC) was evaluated according to the method described by Ivanov et al. (2014). An aliquot of 1 ml of the plant extract was added to 0.1 ml of 10% $Al(NO_3)_3$, 0.1 ml of 1 M CH_3COOK (Sigma-Aldrich, Merck), and 3.8 ml of distilled water. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm. Quercetin was used as a standard, and the results were expressed as mg quercetin equivalents (QE)/g of dw sample.

2.2.5 Antioxidant activity

DPPH radical scavenging assay

The reaction mixture containing 2.85 ml of DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Merck) and 0.15 ml of the tested plant extract was incubated at 37°C for 15 min. The absorbance was measured at 517 nm against a blank (methanol). The antioxidant activity was expressed as mM Trolox equivalents (TE)/g of dw sample.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was freshly prepared with 300 mM acetate buffer with pH 3.6, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM Iron (III) chloride hexahydrate (Sigma-Aldrich, Merck) in distilled water in a ratio of 10:1:1. The reaction mixture (3 ml of FRAP reagent and 0.1 ml of the plant extract) was incubated at 37°C for 10 min, in darkness. The absorbance was measured at 593 nm against a blank (distilled water). The antioxidant activity was expressed as mM TE/g of dw sample (Ivanov et al., 2014).

2.2.6 Antimicrobial activity

The antimicrobial activity of the plant extracts was determined by the agar well diffusion method. (Tumbarski et al., 2018). In the first stage of the experiment, bacterial, yeast, and fungal inocula were prepared. A bacterial counting chamber Thoma (Poly-Optik, Germany) was used for determination of the viable cells and fungal spore counts, after that their final concentrations for inoculation were adjusted to 10^8 cfu/ml (for bacterial/yeasts cells) and to 10^5 cfu/ml (for fungal spores). In the second stage, LBG/MEA media, preliminarily melted and tempered at 45–48°C, were inoculated with the bacterial/yeasts/fungal inocula. Next, the inoculated media were transferred in quantity of 18 ml in sterile Petri dishes (d = 90 mm) (Gosselin™, France), and allowed to harden for 1–2 h. The extracts were pipetted in duplicates of 60 µL into preliminarily prepared wells (d = 6 mm) in the agar media. The Petri dishes were incubated under identical conditions according to the type of microorganism.

After incubation for 24 or 48 hours, the antimicrobial activity of the extracts was determined by measuring the diameter of the inhibition zones (IZ) around the agar wells. Sensitive were considered microorganisms with a diameter of IZ of 18 mm or more (high antimicrobial activity); moderately sensitive were those in which the diameter of IZ was between 12 and 18 mm (moderate antimicrobial activity); resistant were those in which the IZ were up to 12 mm (low antimicrobial activity) or completely missing.

2.2.7 Anti-inflammatory activity

Inhibition of Albumin Denaturation

The anti-denaturation assay was carried out as described by Milusheva et al. (2023a; 2023b). The reaction mixture contained 0.5 mL of a 5% aqueous solution of human albumin (Albunorm 20, Octapharma (IP) SPRL, 1070 Anderlecht, Belgium) and 0.2 mL of the tested extracts, which were diluted in DMSO at a concentration of 10 mg/ml. The samples were incubated at 37ºC for 15 minutes. Each tube was filled with 2.5 mL of phosphate-buffered saline (pH 6.3), heated for 30 minutes to 80°C, and then cooled for 5 minutes. The turbidity of the samples was measured spectrophotometrically at 660 nm (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA 95051, USA). A mixture of 2.5 mL of buffer and 0.2 mL of DMSO was used for the blank, while the product control contained 0.5 mL of serum albumin and 2.5 mL of buffer.

The percent inhibition of protein denaturation was calculated according to the formula (1):

$$\text{Percentage of inhibition danaturation} = \frac{(Absorbance\ control - Absorbance\ sample)}{Absorbance\ control} \times 100 \quad (1)$$

The control represented 100% protein denaturation. Commercially available anti-inflammatory drugs were used for comparison. Their anti-inflammatory effect was determined as a percentage of inhibition of albumin denaturation, following the same protocol as for the novel compounds.

2.2.8 Statistical analysis

Data from triplicate experiments were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (±SD) and maximum estimation error at significance levels $p < 0.05$ (MS Office Excel 2010).

3. Results and Discussion

3.1 Physicochemical characteristics

The results of the physicochemical analysis of the three research plants are presented in Table 2. The results showed that blackberry, aronia, and mint have similar moisture values. Aronia berries had the highest total carbohydrate content (18.26%), which was about twice that of blackberry and mint leaves. Blackberry had the highest protein content (10.20%), followed by mint leaves (8.95%), and aronia berries had the lowest content (6.46%). In terms of vitamin C content, blackberry and aronia showed the same relatively high values (1551 mg/100 g dw), while mint contained almost twice less (846 mg/100 g dw).

Other authors have also determined the physicochemical characteristics. Cervenka et al. (2011) reported a protein content of 3.7 g/100 g DM in the fruit. Compared to this value, the protein content in the present study was 1.5–2 times higher, reaching 6.46%. Ochmian et al. (2012) found that aronia berries contained 15.3–19.5% dry matter and also reported a higher dry matter content in other berry samples (26.67–30.76%). Ochmian et al. (2012) found that aronia berries contained 15.3–19.5% dry matter, and also reported a high dry matter content in the berries (26.67–30.76%). Compared to the results obtained by the scientific research team, where the dry matter of aronia was 91.55%, this value was 3.5–4 times less. Zieniewska et al. (2020) determined the amount of carbohydrates in aronia, obtaining a result of 12.7%, which was much less than the results obtained in our study. The amount of proteins determined by the same authors was 0.7%, which was much less than the results obtained in the research done by our team (6.46%).

3.2 Total phenolic content, total flavonoid content, and antioxidant activity

The number of total phenolics (TPC) and flavonoids (TFC) of methanolic extracts of mint leaves and the two fruits of blackberry and aronia, were measured. The results are presented in Table 3. The highest TPC and TFC values were found in the mint leaf extract, 6.86 mg GAE/g dw and 2.17 mg GAE/g dw, respectively. High anthocyanin content was found in aronia and blackberry extracts with values of 180.52 mg cyd-3-glu/g dw and 190.45 mg cyd-3-glu/g dw, respectively. The antioxidant activity was also measured by two methods – radical-scavenging ability (DPPH) and ferric-reducing antioxidant power assay (FRAP). The results also showed a high correlation between the DPPH and FRAP assay with TPC – r^2 =0.7793 and r^2 =0.9894. Consequently, the TPC contributed to the antioxidant properties of the tested samples. Regarding the content of anthocyanins, Memete et al. (2023) found that the anthocyanin in the blackberries of the variety „Spineless“ was 329.26 ± 9.36 mg/g dw, which was much higher compared to our obtained result, 191.45 ± 0.05 mg/g dw. In comparison, ‚Loch Ness‘ and ‚Thorn Free‘ fruits showed total phenolic content of 1830.98 ± 13.55 and 1687.14 ± 62.41 mg GAE/100 g dw, respectively. The amount found by Memete et al. (2023) was several times greater than that found in our study, 6.86 ± 0.02 mg GAE/g dw. Different author groups found the following content of polyphenols in the berries of aronia: 7849 mg/100 g DM (fresh mass) (Oszmianski and Wojdy-

Table 2. Physicochemical characteristics of blackberry, aronia, and mint

Dried plant material	Moisture, %	Dry matter, %	Total ash, %	Carbo-hydrates, %	Proteins, %	Vitamin C, mg/100 g
Fruits						
Blackberry	9.12 ± 0.03	90.88 ± 0.03	4.53 ± 0.00	10.64 ± 0.41	10.20 ± 0.14	1551 ± 4.6
Aronia	8.45 ± 0.03	91.55 ± 0.02	1.87 ± 0.00	18.26 ± 0.71	6.46 ± 0.09	1551 ± 4.6
Leaves						
Mint	10.05 ± 0.03	89.95 ± 0.03	6.83 ± 0.00	9.37 ± 0.41	8.95 ± 0.10	846 ± 2.5

Source: Authors' own elaboration

lo, 2005); 6351.38 mg/100 g DM (Teleszko and Wojdyło, 2015); 37.600 mg/kg DM (Hudec et al., 2006); 1079–2996 mg gallic acid equivalents/100 g fresh weight (Wangensteen et al., 2014); 819–1330 mg GAE/100 g FW (fresh weight) (Kulling and Rawel, 2008); 778–1285 g GAE/kg FW (Jing et al., 2008). The amount of polyphenols found in aronia berries in our study was 400.00 ± 0.02 times less (mg GAE/100 g dw). It is assumed that the difference is because our raw material was dried, and the scientific teams cited above work with fresh mass. The drying process reduced biologically active components, total phenols, and flavonoids, which inevitably decreased the antioxidant activity. However, the plant material remained a rich source of bioactive compounds. Tafrihi et. al. (2021) found that naturally dried *M. longifolia* extract had higher phenolics (113.8 mg GAE/g) and flavonoids (106.7 mg RTE/g) than laboratory oven-dried samples. Researchers reported that of nine *Mentha species*, *M. longifolia* was the most effective, demonstrating 88.6% antioxidant activity. Compared to the results obtained by us, which were by DPPH 53.75 ± 2.31 (mM TE/g dw), by FRAP 55.41 ± 3.44 (mM TE/g dw), the considered species showed better antioxidant activity. Many factors are responsible for these results, such as cultivation methods of wild plant harvest, the type of laboratory research, and extraction methods. The plant raw material that our team used also grown wild.

Table 3. Total phenolics, flavonoids, anthocyanins, and antioxidant activity of methanolic blackberry, aronia, and mint extracts

Extract	Total phenols (mg GAE/g dw)	Total flavonoids (mg QE/g dw)	Total anthocyanins (mg cyd-3-glu/g dw)	Antioxidant activity	
				DPPH (mM TE/g dw)	FRAP (mM TE/g dw)
Fruits					
Blackberry	6.12 ± 0.05	1.10 ± 0.01	191.45 ± 0.05	75.15 ± 0.06	51.79 ± 0.03
Aronia	4.00 ± 0.02	1.52 ± 0.02	180.52 ± 0.03	23.54 ± 0.10	24.14 ± 0.02
Leaves					
Mint	6.86 ± 0.02	2.17 ± 0.02	0.00 ± 0.00	53.75 ± 2.31	55.41 ± 3.44

Source: Authors' own elaboration

Table 4. Antimicrobial activity of methanolic blackberry, aronia, and mint extracts

Test microorganism	Plant material (inhibition zones, mm)		
	Aronia fruits	Blackberry fruits	Mint leaves
B. subtilis ATCC 6633	9 ± 0.00	12 ± 0.00	10 ± 0.00
B. amyloliquefaciens 4BCL	–	8 ± 0.00	–
B. cereus NCTC 11145	–	12 ± 0.00	10 ± 0.00
S. aureus ATCC 25923	8 ± 0.00	10 ± 0.00	–
L. monocytogenes NBIMCC 8632	8 ± 0.00	8 ± 0.00	8 ± 0.00
E. faecalis ATCC 19433	8 ± 0.00	–	9.5 ± 0.71
M. luteus 2YC-YT	9.5 ± 0.71	18.5 ± 0.71	13.5 ± 0.71
S. enteritidis ATCC 13076	8 ± 0.00	–	10 ± 0.00
S. typhimurium NBIMCC 1672	–	10 ± 0.00	9 ± 0.00
K. pneumoniae ATCC 13883	–	8 ± 0.00	9 ± 0.00
E. coli ATCC 25922	10 ± 0.00	11 ± 0.00	10 ± 0.00
P. vulgaris ATCC 6380	–	12 ± 0.00	–
P. mirabilis 56/10	–	10 ± 0.00	–
P. aeruginosa ATCC 9027	–	12 ± 0.00	9.5 ± 0.71
C. albicans NBIMCC 74	–	10 ± 0.00	–
S. cerevisiae ATCC 9763	8 ± 0.00	8 ± 0.00	–
A. niger ATCC 1015	–	–	–
A. flavus	–	–	–
P. chrysogenum	9 ± 0.00	–	–
Rhizopus sp.	8 ± 0.00	8 ± 0.00	–
F. moniliforme ATCC 38932	–	–	–
Mucor sp.	–	–	–

Source: Authors' own elaboration

3.3 Antimicrobial activity

The results from the antimicrobial activity test are presented in Table 4.

Dried aronia extract showed a limited antibacterial activity (IZs 8 – 10 mm) against the test microorganisms *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *L. monocytogenes* NBIMCC 8632, *E. faecalis* ATCC 19433, *M. luteus* 2YC-YT, *S. enteritidis* ATCC 13076, and *E. coli* ATCC 25922. The extract demonstrated weak antifungal effect (IZs 8 – 9 mm) on the yeast *S. cerevisiae* ATCC 9763, and the fungi *P. chrysogenum* and *Rhizopus* sp.

Dried blackberry extract exhibited the highest inhibitory activity against *M. luteus* 2YC-YT. In contrast, the extract showed moderate antibacterial activity (IZs ≥ 12 mm) against *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *P. vulgaris* ATCC 6380, and *P. aeruginosa* ATCC 9027, and low antibacterial activity (IZs 8 – 11 mm) against *B. amyloliquefaciens* 4BCL, *S. aureus* ATCC 25923, *L. monocytogenes* NBIMCC 8632, *S. typhimurium* NBIMCC 1672, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, and *P. mirabilis* 56/10. Similar to aronia, blackberry extract demonstrated weak antifungal potential (IZs 8 – 10 mm) against the yeasts *C. albicans* NBIMCC 74 and *S. cerevisiae* ATCC 9763, and the fungal strain *Rhizopus* sp.

Mint extract possessed the highest inhibitory activity against *M. luteus* 2YC-YT (IZ = 13.5 mm). Weak inhibitory effect (IZs 8 – 10 mm) on *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *L. monocytogenes* NBIMCC 8632, *E. faecalis* ATCC 19433, *S. enteritidis* ATCC 13076, *S. typhimurium* NBIMCC 1672, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 9027. Mint extract did not inhibit the growth of any of the yeasts and fungal strains tested.

3.4 In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activity assay was evaluated as an inhibition of albumin denaturation, assessing the degree of resistance to denaturation of the human albumin molecule in the presence of extracts. The human albumin anti-denaturation method evaluated the anti-inflammatory properties of the two berries extracts and one leaf extract (Figure 1). All extracts protected the human albumin against thermal denaturation and exhibited anti-inflammatory activity. Mint leaf extract showed the best anti-inflammatory activity, followed by blackberry and aronia berries (Figure 1).

Non-steroidal and steroidal anti-inflammatory drugs affect inflammation by reducing it. However, these drugs have many adverse side effects. Anti-inflammatory drugs have showed a dose-dependent ability to inhibit thermally induced protein denaturation (Manolov et al., 2023).

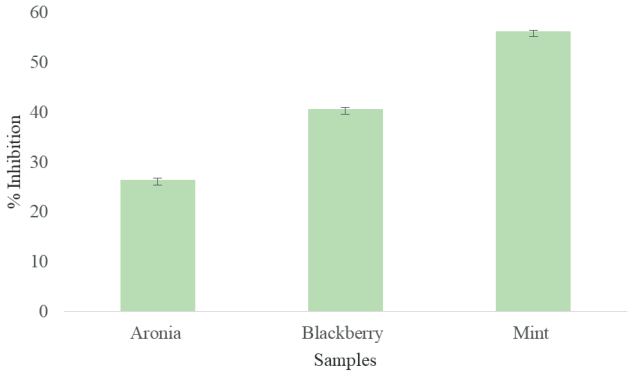

Fig. 1. Percent inhibition of albumin denaturation of fruit and leaf extracts

Source: Authors' own elaboration

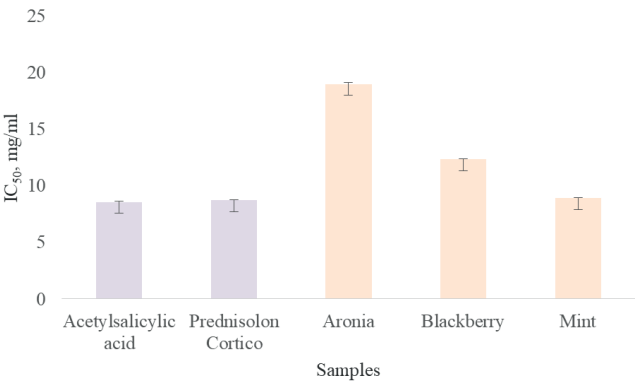

Fig. 2. Inhibition of albumin denaturation of the controls, fruit and leaf extracts, expressed as IC_{50}

Source: Authors' own elaboration

The anti-inflammatory activity of the extracts was compared with two commercial anti-inflammatory drugs (acetylsalicylic acid and Prednisolon Cortico) used as standards. In Figure 2, the results are presented as half maximal inhibitory concentration (IC_{50}).

The mint sample showed similar activity to the two standards used. Blackberry and aronia berries extracts showed lower anti-inflammatory activity than the standards, 1.5 times and 2 times, respectively. The data showed that the tested plant extracts showed good protection against albumin denaturation (Figure 2).

4. Conclusion

The studied plant species are natural sources of biologically active compounds with antioxidant, antimicrobial and anti-inflammatory effects. In the course of the study, it

was found that blackberry and aronia contained significant amounts of vitamin C, as well as a high anthocyanins content. The highest values of total phenols (TPC) and total flavonoids (TFC) were found in the mint leaf extract. The results obtained revealed the potential of the studied plant species for application in pharmaceutical and functional food products.

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5. Conflict of interest

The authors declare no conflicts of interest.

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