Physicochemical characteristics and biological potential of the fruits of four medicinal plants from Dospat region, Bulgaria

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Abstract. This study aimed to investigate the physicochemical characteristics and biological potential of the fruits (dried and frozen) of four wild growing medicinal plants from Dospat region, Rhodope Mountain, Bulgaria – rosehip (*Rosa canina*), black elder (*Sambucus nigra*), cranberry (*Vaccinium vitis-idaea*) and juniper (*Juniperus communis*). The physicochemical properties (moisture, ash, carbohydrates, proteins and vitamin C contents) were determined. Afterwards, the biological activities (total phenolic content - TPC, total flavonoid content - TFC, antioxidant, antimicrobial and anti-inflammatory) of methanolic extracts were investigated. The TPC values varied between 5.37 mg GAE/g dw (dried juniper berry) and 19.64 mg GAE/g dw (frozen cranberry), while TFC values were from 1.26 mg QE/g dw (dried rosehip) to 9.74 mg QE/g dw (frozen elderberry). The values of antioxidant activity determined by the DPPH method were between 24.23 mM TE/g dw (dried juniper berry) and 154.63 mM TE/g dw (frozen cranberry), while those obtained by the FRAP method varied from 23.06 mM TE/g dw (dried juniper berry) to 138.54 mM TE/g dw (frozen cranberry). The cranberry and juniper fruits showed moderate to high antimicrobial activity. The juniper berry and elderberry demonstrated high anti-inflammatory potential. The results revealed the great potential of the studied berries for application in pharmaceutical and functional food products.

1 Introduction

Over the last decades, extensive research attention has been paid to the medicinal and culinary plants as rich sources of nutrients and biologically active compounds, which provide diverse health benefits, thus finding wide application in the food, pharmaceutical and cosmetic industries. In this regard, the increasing use of the plants in various branches of the industry, in particular their growing utilization in the development of innovative functional foods, alternative medical formulations and different herbal supplements, requires more in-depth and extensive knowledge of their chemical composition and biological properties.

Rosehip (*Rosa canina* L.) is a perennial shrub from the *Rosaceae* family, natively growing on rocky and sloping sites. It is resistant to severe environmental conditions and often grows on poor soils with limited water access [1, 2]. Rosehip fruits are well known as rich sources of vitamins, mainly ascorbic acid (vitamin C), B1, B2, PP, A, E and K, carotenoids, polyphenolic compounds and polysaccharides. Rosehip fruits also contain significant amounts of minerals - macroelements (P, K, Ca, Mg) and microelements (Fe, Cu, Mn, Zn). The carotenoids presented in rosehip fruits are frequently used as natural food colorants. In the folk and traditional medicine, rosehip fruits are used due to their healing properties, such as antioxidant, antimicrobial, anti-inflammatory, diuretic, spasmolytic, vasodilatory, anticarcinogenic, anti-aging, anti-allergic, sedative, antitussive, antitoxic, nutritive and general strengthening effects [3-5].

Black elder (*Sambucus nigra* L.) is a shrub belonging to the *Adoxaceae* family, widely spread as a part of the spontaneous flora of Europe, Asia, North Africa and America, growing at various altitudes, preferably in sunny places and on alkaline soils. Since antiquity, all parts of this shrub have been used as a food ingredients and for medicinal purposes; however recent studies revealed that all parts of the plant, except the flowers and ripe fruit (elderberry) contain a poisonous cyanogenic glycoside called sambunigrin, causing poisonings in men and animals [6]. The chemical analyses demonstrated that elderberries comprise large amounts of anthocyanins as predominant phytocomponents, followed by other

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polyphenols, such as flavonols and proanthocyanidins [7]. Elderberries contain also high concentrations of organic acids, sugars, vitamin C and minerals (K, P, Ca, Mg) [8]. European elderberries are characterized with a broad spectrum of pharmacological activities, such as antioxidant, antimicrobial, antiviral, immunomodulatory, anti-inflammatory, neuroprotective, cardioprotective, anticancer, antidiabetic among the others [9, 10].

Cranberry (*Vaccinium vitis-idaea*) also known as lingonberry, partridgeberry or cowberry, is an evergreen shrub from the *Ericaceae* family. The cranberry fruits are abundant in phenolics, especially anthocyanins, which contribute to their significant antioxidant potential and diverse health benefits – diuretic, antimicrobial, anti-inflammatory, neuroprotective and antitussive [11]. In recent years, cranberry fruits are often recommended as a natural antiseptic for the urinary tract as well as for improving digestive tract functions and decreasing the obesity and related cardiovascular disorders [12, 13]. In addition to the mentioned healing properties, anti-fever, reparative, detoxifying, sedative and analgesic effects of the cranberry fruits have also been reported [14]. It was established that certain phenolic fractions of leaves and fruits possessed anticarcinogenic effect by reduction the viability of some cancer cells, such as those of the malignant melanoma, colon adenocarcinoma and renal carcinoma cell lines [15].

Juniper (*Juniperus communis* L.) is an evergreen, perennial coniferous aromatic shrub or tree from the *Cupressaceae* family, with the largest distribution of the woody plant species in the temperate climatic zone of the Northern Hemisphere and possessing great abilities for ecological adaptation [16, 17]. Juniper berries contain multiple biologically active compounds, such as monoterpenes, sesquiterpenes, proteins, sugars, pectin, organic acids, ascorbic acid (vitamin C), wax, gum and camphor [18] that determine their anti-inflammatory, antimicrobial, antioxidant, antidiabetic, hepatoprotective, renal, neuroprotective, and anticarcinogenic properties. Juniper berries extracts and essential oil also find application in the treatment of acute and chronic respiratory, circulatory and rheumatic disorders as well as hypertension, skin infections and others [19, 20].

The largest mountain in Bulgaria, Rhodope Mountain, is situated in the southern part of the country and is separated into two regions: the Eastern (with an average elevation of 329 m above sea level) and the Western (with an average elevation of 1098 m above sea level). The large climatic and soil diversity determine a wide variety of flora in the Rhodope Mountain, on which territory over 2000 species of vascular plants have been found. In the Eastern Rhodopes, the flora is presented mainly by low-stemmed sub-Mediterranean species, while the Western Rhodopes include forests of coniferous species, shrubs and alpine meadows. A large part of the Rhodope Mountain is included in the European network of protected areas "Natura 2000" [21].

Rosehip, black elder, cranberry and juniper represent multi-purpose plants widely used in the pharmaceutical, food and cosmetic industries. Therefore, the present research aimed to study the physicochemical properties, antioxidant activity, antimicrobial and anti-inflammatory effects of the fruits of these four wild growing plants in the Dospat region, Rhodope Mountain, Bulgaria, and to contribute to the limited information on the plants in this area and in general, and their therapeutic applications.

2 Materials and methods

2.1 Materials

2.1.1 Plant material

In the present study, the fruits of four wild growing plants – rosehip (*Rosa canina* L.), black elder (*Sambucus nigra* L.), cranberry (*Vaccinium vitis-idaea* L.) and juniper (*Juniperus communis* L.) were analysed. The berries were harvested in the period June–September 2024 in the region of Dospat, Smolyan district (41°66′N 24°16′E), located in the Western Rhodope Mountain, Bulgaria. The raw plant material was identified according to the Herbarium Academiae Scientiarum Bulgariae. The four types of berries were delivered to the laboratory fresh, thereafter each of them was divided into two portions – the first was freezed (-15°C), while the second was air-dried at room temperature for further analyses (Fig. 1).

Fig. 1. Frozen, ground and dried fruit material used in the study.

2.1.2 Test microorganisms

Twenty-two microorganisms including seven Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus amyloliquefaciens* 4BCL-YT, *B. 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 and Biotechnology at the University of Food Technologies, Plovdiv, Bulgaria were selected for the antimicrobial activity test.

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 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 was dissolved in 1 L of deionized water, pH 5.4 ± 0.2.

Both culture media were prepared in accordance with the manufacturer's instructions (Scharlab SL, Spain) and autoclaved at 121°C for 20 min prior to use.

2.2 Methods

2.2.1 Extracts preparation

Using a blender, the dried and frozen berries were initially ground. Each powdered sample weighed 4 g, which were then macerated with 40 ml of methanol (Sigma-Aldrich, Merck, Germany). The samples were left at room temperature for 48 h in the dark after being stirred for 10-15 s using a vortex (V-1, Biosan, Latvia). After filtration through filter paper, the extracts were kept at 4°C until analyses. Next, the extracts were divided into two parts, and the second part was vacuum-evaporated to remove the methanol and redissolved in dimethyl sulfoxide - DMSO (Sigma-Aldrich, Merck, Germany) to assess the anti-inflammatory efficacy [22].

2.2.2 Physicochemical analyses

Physicochemical characteristics of the fruits of four plants were assessed in accordance with the Bulgarian State Standards: moisture content - BSS ISO 939:2021 [23], ash content - BSS ISO 928:2004 [24], carbohydrates - BSS 7169:1989 [25], proteins - BSS 15438:1982 [26] and ascorbic acid (vitamin C) - BSS 11812:1991 [27].

2.2.3 Total phenolic content

The total phenolic content (TPC) was determined using a Folin-Ciocalteu reagent by the method of Ivanov et al. [28]. The results were expressed as mg equivalent of gallic acid (GAE)/g of dry weight (dw) sample.

2.2.4 Total flavonoid content

The total flavonoid content (TFC) was evaluated according to Ivanov et al. [28]. The results were expressed as mg quercetin equivalents (QE)/g of dw sample.

2.2.5 Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity using the DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) was evaluated according to Ivanov et al. [28]. The results were expressed as mM Trolox equivalents (TE)/g of dw sample.

Ferric-reducing antioxidant power (FRAP) assay

The antioxidant activity using the FRAP reagent was assessed as previously described by Ivanov et al. [28]. The results were expressed as mM TE/g of dw sample.

2.2.6 Antimicrobial activity

The antimicrobial activity of the methanolic fruit extracts was determined by the standard agar well diffusion method [29]. After the incubation (24/48 h), the inhibitory effect was determined by measuring the inhibition zone (IZ) diameter around the wells. The antimicrobial activity was assessed as follows: high (IZ diameter ≥ 18 mm); moderate (IZ diameter 12 - 18 mm); low (IZ ≤ 12 mm).

2.2.7 In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory activity assay was performed according to Milusheva et al. [30]. The results were expressed as percentage of inhibition of albumin denaturation and as a half of the maximal inhibitory concentration (IC_{50}). As controls, commercially available steroid (Prednisolone) and non-steroid (Aspirin) anti-inflammatory drugs at the same concentrations were used.

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 $p < 0.05$ [29].

3 Results and discussions

3.1 Physicochemical characteristics

The results from the physicochemical analyses showed that the frozen fruits had higher moisture content and lower dry substance content compared to the dried fruit material, respectively. The ash content of the four fruits was almost equal in both frozen and dried fruit material. The carbohydrate, protein and ascorbic acid (vitamin C) contents of the frozen fruit material were higher in comparison with those of the dried fruits (Table 1).

Vasilj et al. [31] performed a comparative analysis of fresh and dried wild rosehip fruits (*R. canina* L.) from different regions of Bosnia and Herzegovina. The fresh rosehip fruits were characterized by moisture content ranging from 44.89 to 47.01 %, total acid content - from 31.00 to 45.10 % and total ash content - from 2.15 to 2.41 %, which findings were similar to our results. Ivanova et al. [1] determined that vitamin C content of wild rosehip from Bulgaria was 245.6 mg/100 g dw vs. 153.8 mg/100 g dw for the cultivated type of rosehip, which was significantly lower than our data. The protein content had values of 7.38 % and 1.6 % for the wild and cultivated rosehip, respectively.

A study conducted by Domínguez et al. [32] showed that fresh (frozen) elderberry (*S. nigra*) from Spain contained 78.91 % moisture, 1.02 % ash and 2.97 % proteins. The results for the moisture and ash contents of the Spanish elderberry were similar to ours; however the sample exhibited lower protein value, compared to our samples. Kolarov et al. [33] examined samples of fresh wild growing elderberry from Serbia and determined that dry solid was 23.6 % (close to our result), sugar content was 5.17 %, while the value of ascorbic acid was 0.3 mg/g (lower than our data).

Odabaş-Serin and Bakir [34] investigated ripe juniper berries of *Juniperus drupacea* L. collected from two provinces in Turkey - Kahramanmaraş and Adana. The authors obtained the following results - total dry matter of 92.89 % and 93.3 %, ash content of 4 % and 3.38 %, protein content of 2.06 % and 3.74 % for the samples of Kahramanmaraş and Adana provinces, respectively. Akinci et al. [35] reported values of total dry matter of 76.57 %, ash of 2.51 % and protein content of 2.45 % for juniper berries of *J. drupacea* L. from the village of Akseki, near Antalya, Turkey. Both authors' teams reported results close to these in our study.

Table 1. Physicochemical characteristics of the fruits of four medicinal plants.

Parameter	Fruit material							
	Rosehip		Elderberry		Cranberry		Juniper berry	
	Frozen	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*
Moisture, %	45.52 ± 0.11	13.28 ± 0.02	78.15 ± 0.18	5.8 ± 0.02	78.31 ± 0.19	13.06 ± 0.04	20.90 ± 0.06	8.81 ± 0.03
Dry substa-nce, %	54.48 ± 0.11	86.72 ± 0.02	21.85 ± 0.18	94.2 ± 0.02	21.69 ± 0.18	86.94 ± 0.02	79.10 ± 0.18	91.19 ± 0.02
Ash, % dw	2.99 ± 0.00	2.95 ± 0.00	3.37 ± 0.00	3.60 ± 0.00	1.33 ± 0.00	1.05 ± 0.00	1.85 ± 0.00	1.91 ± 0.00
Carbohydra-tes, % dw	30.46 ± 0.60	22.34 ± 0.75	32.95 ± 0.30	14.39 ± 0.50	62.7 ± 0.50	37.09 ± 1.29	32.43 ± 1.20	31.11 ± 0.45
Proteins, % dw	4.97 ± 0.06	2.82 ± 0.02	13.18 ± 0.04	5.29 ± 0.07	6.31 ± 0.02	3.88 ± 0.05	4.80 ± 0.06	4.03 ± 0.02
Vitamin C, mg/100 g dw	672.9 ± 1.50	390.2 ± 0.19	400.0 ± 1.50	167.6 ± 0.47	545.8 ± 0.26	207.0 ± 0.54	309.0 ± 0.84	156.7 ± 0.28

3.2 Total phenolic content, total flavonoid content and antioxidant activity

A decrease in the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity values of the dried fruit materials was observed, probably due to degradation of the biologically active compounds during the drying process (Table 2). The frozen cranberry fruit exhibited the highest TPC value (19.64 mg GAE/g dw), whereas the black elder fruit showed the highest TFC value (9.74 mg QE/g dw). The extract of frozen cranberry fruit demonstrated the highest values of antioxidant activity determined by two independent methods - DPPH and FRAP (154.63 and 138.54 mM TE/g dw, respectively), followed by the extracts of frozen rosehip (129.18 mM TE/g dw) and black elder fruits (122.88 mM TE/g dw), determined by FRAP method.

Paunović et al. [36] investigated the composition of rosehip (*Rosa canina* L.) from Serbia and concluded that the desiccation process leads to a decrease of bioactive components, such as ascorbic acid, total phenolics and flavonoids, which inevitably reduces the antioxidant activity; however the plant material still remains a rich resource of bioactive compounds. The same lowering trend after drying process was confirmed by our results. Peña et al. [37] investigated the biologically active compounds and antioxidant potential of rosehip (*R. canina* L. and *Rosa rubiginosa* L.) from areas in Southern Chile. Vitamin C and flavonols were identified as the main bioactive compounds. The results showed a linear correlation between the concentration of flavonols, catechins and antioxidant activity (determined by the

TEAC, CUPRAC and DPPH methods) of the studied rosehip. Murathan et al. [38] examined four different rosehip species from the eastern Anatolia region of Turkey and found that TPC varied between 1081 and 6928 mg GAE/100 g, while the total anthocyanins ranged from 40.10 to 56.70 %. The antioxidant activity (by the FRAP assay) among the investigated cultivars ranged widely - from 10.04 to 97.95 mmol TE/g. Bilgin et al. [39] investigated nine rosehip genotypes (*Rosa* spp.) from different regions of Turkey. The biological potential was assessed by the TPC and the antioxidant activity. The nine rosehip genotypes exhibited similar TPC values ranging from 303.59 to 347.97 mg GAE/100 g, while the antioxidant activity (FRAP method) showed significant differences, ranging from 25.07 to 195.43 µmol TE/g. Benković-Lačić et al. [40] investigated the chemical profile and biopotential of different genotypes of wild and cultivated rosehip from Slavonia region, Croatia. The TPC values varied within narrow ranges between the genotypes - 4033.37 - 4634.43 mg GAE/100 g, while the antioxidant activity (DPPH method) ranged from 69.11 to 86.79 %. Roman et al. [41] examined the TPC, TFC and antioxidant potential of rosehip biotypes from the region of Transylvania, Romania. The authors found that the TPC and TFC ranged from 326 to 575 mg/100 g and from 101.3 to 163.3 mg/100 g, respectively. Antioxidant activity (DPPH assay) was between 63.35 and 127.8 µM TE/100 g. In another study, Eroğul and Oğuz [42] tested naturally growing rosehip from the Adiyaman region, Turkey and found that TPC ranged from 3.62 to 5.42 GAE/g dw, whereas antioxidant activity (FRAP method) ranged between 56.80 and 113.60 µmol TE/g dw.

Black elder (*Sambucus nigra* L.) is known as a rich source of phenolic acids, anthocyanins, flavonoids, chlorophyll and carotenoids. The phenolic compounds are presented mostly in the flowers; the carotenoids, chlorophyll and flavonoids are concentrated in the leaves, while the fruits contain significant amounts of anthocyanins [43]. Ozola and Dūma [44] performed a study on the antioxidant content of black-colored fruits (blackberry, blueberry, elderberry and blackcurrant), and found that elderberry dominated over the other studied fruits in terms of the amount of total phenolics (537.96 mg GAE/100 g fw), flavonoids (112.65 mg QE/100 g fw) and anthocyanins (161.51 mg/100 g fw). Haş et al. [45] confirmed the prevalence of anthocyanins (41.8 %) in the phenolic fraction of an extract of elderberry. The major anthocyanins in the extract were cyanidin-sambubiosides and cyanidin-glucosides, accounting 90.1 % of the total anthocyanin content (TAC). The highest antioxidant activity value (185 µmol Fe^{2+}/g dw) was determined by the FRAP assay. Kolarov et al. [33] investigated the chemical composition, phenolic compounds and antioxidant activity of fresh elderberry growing wild in Serbia, which showed TPC of 17.40 mg GAE/g fw, TFC of 8.09 mg QE/g fw and TAC of 5.37 mg C_3G/g fw. The values of antioxidant activity were 125.03 mg TE/g fw (DPPH method), 51.46 mg TE/g fw (FRAP method) and 36.98 mg TE/g fw (ABTS method).

Dróżdż et al. [46] examined two types of extracts (ethanolic and ethyl acetate) of wild growing cranberry (*V. vitis-idaea* L.) from four regions in Poland. The authors stated that TPC ranged from 468 to 661.1 mg GAE/100 g fw (ethanolic extract) and from 458.2 to 594.0 mg GAE/100 g fw (ethyl acetate extract), which values were lower in comparison with our results. The TFC values expressed as catechin equivalents were in the range of 1.12–1.79 µg CE/100 g fw (ethanolic extract) and 1.28–1.49 µg CE/100 g fw (ethyl acetate extract). Similar results were obtained by Lee and Finn [47], who detected TPC values of five cranberry (lingonberry) cultivars grown in Oregon (USA) ranging between 431 and 660 mg/100 g fw. Oszmiański et al. [48] investigated the phytochemical composition and antioxidant activity of six cranberry cultivars (*Vaccinium macrocarpon* L.) from Poland. Three of them (Howes, Franklin and Stevens) were characterized by the highest TPC, ranging from 3584 mg/100 g dw to 4219 mg/100 g dw, and exhibited strong antioxidant activity tested by three methods: ABTS (226-264 µmol TE/g dw), FRAP (102-139 µmol TE/g dw), DPPH (235-320 µmol TE/g dw). A similar study was conducted by Borowska et al. [49], who examined five cranberry cultivars and one wild cranberry species (*Vaccinium oxycoccus*) grown in Poland. The authors found that the TPC ranged from 192.1-374.7 mg/100 g fw, whereas the anthocyanins were from 43.3 to 77.2 mg/100 g fw, respectively. The antioxidant potential was also determined, which showed values of 33.9-68.8 µmol TE/g fw (by the DPPH assay) and 9.3-16.4 µmol TE/g fw (by the ABTS).

Miceli et al. [50] investigated a methanolic extract of juniper (*Juniperus drupaceae* Labill.) from Turkey and found high levels of total phenolics (48.06 mg GAE/g extract), which were presented mainly by phenolic acids. The extract exhibited high antioxidant activity (IC_{50} = 0.38 mg/ml) determined by the DPPH method. High antioxidant potential of aqueous and ethanolic extracts of common juniper (*J. communis* L.) was also detected by Elmastaş et al. [51], who established that aqueous and ethanolic extracts with concentrations of 20, 40 and 60 µg/ml had the ability to inhibit peroxidation (75% to 92%) of linoleic acid emulsion. Similarly, Bacém et al. [52] found that the TPC of juniper fruit (*J. communis* L.) ranged from 11.13 to 16.16 mg GAE/g, which values were higher compared to our results. The authors also stated that the TPC in the leaf samples was significantly higher (141.12 mg GAE/g) than those of the berries. Fierascu et al. [20] studied a hydroalcoholic extract of a wild growing *J. communis* L. from Romania and determined that TPC value was 19.23 mg GAE/100 g of extract, whereas the TFC expressed in rutin equivalents was 5109.6 mg RE/100 g of extract. The antioxidant potential of the juniper extract was estimated to be 81.63 % (measured by the DPPH method).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AO) of the methanolic fruit extracts.

Parameter	Fruit material							
	Rosehip		Elderberry		Cranberry		Juniper berry	
	Frozen	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*
TPC, mg GAE/g dw	12.53 ± 0.12	5.54 ± 0.18	18.17 ± 0.02	8.42 ± 0.22	19.64 ± 0.06	8.67 ± 0.21	9.88 ± 0.15	5.37 ± 0.03
TFC, mg QE/g dw	2.60 ± 0.01	1.26 ± 0.05	9.74 ± 0.02	4.52 ± 0.15	5.07 ± 0.01	2.40 ± 0.09	7.13 ± 0.21	3.65 ± 0.05
AO, DPPH (mM TE/g dw)	98.89 ± 0.45	41.54 ± 1.11	115.19 ± 0.37	53.44 ± 0.67	154.63 ± 0.84	59.56 ± 1.52	45.32 ± 1.12	24.23 ± 0.04
AO, FRAP (mM TE/g dw)	129.18 ± 3.88	36.48 ± 2.31	122.88 ± 0.27	31.90 ± 0.59	138.54 ± 0.18	59.27 ± 1.49	57.29 ± 1.02	23.06 ± 0.02

3.3 Antimicrobial activity

Screening for antimicrobial activity (Table 3) revealed that the methanolic extracts of frozen and dried fruits exhibited varying degrees of antibacterial activity, but limited antifungal activity. The results demonstrated that the extracts of frozen and dried rosehip had low inhibitory activity against *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *L. monocytogenes* 8632, *E. faecalis* ATCC 19433, *S. enteritidis* ATCC 13076, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 9027. Only the extract of dried rosehip showed low antimicrobial effect on *S. typhimurium* 1672 and the fungus *P. chrysogenum*, and moderate effect on *K. pneumoniae* ATCC 13883. Both types of rosehip extracts exhibited moderate antimicrobial effect on *M. luteus* 2YC-YT.

Table 3. Antimicrobial activity of the methanolic fruit extracts.

Parameter	Fruit material (inhibition zones, mm)							
	Rosehip		Elderberry		Cranberry		Juniper berry	
	Frozen	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*	*Frozen*	*Dried*
B. subtilis ATCC 6633	8 ± 0.00	10 ± 0.00	8 ± 0.00	9 ± 0.00	8 ± 0.00	12 ± 0.00	15 ± 1.41	19 ± 1.41
B. amylolique-faciens 4BCL	-	-	8 ± 0.00	-	8 ± 0.00	10 ± 0.71	14 ± 0.00	12 ± 0.71
B. cereus NCTC 11145	9 ± 0.00	10 ± 0.00	9 ± 0.00	10 ± 0.00	9 ± 0.71	13 ± 0.71	20 ± 0.71	23 ± 1.41
S. aureus ATCC 25923	-	-	-	-	-	10 ± 0.00	15 ± 0.00	13 ± 0.00
L. monocyto-genes 8632	8 ± 0.00	9 ± 0.71	-	-	8 ± 0.00	13 ± 0.71	15 ± 0.71	16 ± 1.41
E. faecalis ATCC 19433	8 ± 0.00	10 ± 0.71	8 ± 0.00	8 ± 0.00	8 ± 0.00	12 ± 0.71	15 ± 0.71	10 ± 0.00
M. luteus 2YC-YT	15 ± 0.00	17 ± 0.71	8 ± 0.00	-	11 ± 0.00	26 ± 1.41	24 ± 1.41	24 ± 2.12
S. enteritidis ATCC 13076	8 ± 0.00	10 ± 0.00	8 ± 0.00	-	8 ± 0.00	12 ± 0.71	13 ± 0.00	10 ± 0.71
S. typhimurium 1672	-	10 ± 0.00	-	-	-	11 ± 1.41	-	10 ± 0.71
K. pneumoniae ATCC 13883	-	13 ± 0.00	-	9 ± 0.00	-	13 ± 0.00	10 ± 0.00	9 ± 0.71
E. coli ATCC 25922	9 ± 0.00	11 ± 0.00	8 ± 0.00	9 ± 0.00	8 ± 0.00	12 ± 0.71	18 ± 0.00	17 ± 0.71
P. vulgaris ATCC 6380	-	-	-	-	-	11 ± 0.00	-	9 ± 0.00
P. mirabilis 56/10	-	-	-	-	-	11 ± 0.71	-	-
P. aeruginosa ATCC 9027	9 ± 0.00	10 ± 0.00	8 ± 0.00	8 ± 0.00	8 ± 0.00	12 ± 0.00	16 ± 0.71	17 ± 0.71
C. albicans NBIMCC 74	-	-	-	-	-	-	-	-

S. cerevisiae ATCC 9763	-	-	-	-	-	-	8 ± 0.00	8 ± 0.00
A. niger ATCC 1015	-	-	-	-	-	-	-	-
A. flavus	-	-	-	-	-	8 ± 0.00	13 ± 0.00	-
P. chrysogenum	-	8 ± 0.00	8 ± 0.00	9 ± 0.00	8 ± 0.00	9 ± 0.71	-	9 ± 0.00
F. moniliforme ATCC 38932	-	-	-	-	-	8 ± 0.00	-	9 ± 0.00
Rhizopus sp.	-	-	-	-	-	8 ± 0.00	10 ± 0.00	8 ± 0.00
Mucor sp.	-	-	-	-	-	-	11 ± 0.00	-

The methanolic extracts of elderberry exhibited limited antimicrobial potential regardless of the condition of the fruit material (frozen or dried). The extract of frozen elderberry had low antimicrobial activity against *B. subtilis* ATCC 6633, *B. amyloliquefaciens* 4BCL, *B. cereus* NCTC 11145, *E. faecalis* ATCC 19433, *M. luteus* 2YC-YT, *S. enteritidis* ATCC 13076, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027 and the fungus *P. chrysogenum*. The extract of dried elderberry showed inhibitory effect on *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *E. faecalis* ATCC 19433, *K. pneumoniae* ATCC 13883, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027 and the fungus *P. chrysogenum*.

The methanolic extract of frozen cranberry showed low antimicrobial activity against *B. subtilis* ATCC 6633, *B. amyloliquefaciens* 4BCL, *B. cereus* NCTC 11145, *L. monocytogenes* 8632, *E. faecalis* ATCC 19433, *M. luteus* 2YC-YT, *S. enteritidis* ATCC 13076, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027 and the fungus *P. chrysogenum*. Interestingly, the extract of dried cranberry showed a higher inhibitory effect compared to that of frozen fruit. The antimicrobial activity of the extract of dried cranberry was expressed against all Gram-positive and Gram-negative bacteria tested in the study (at the highest degree against *M. luteus* 2YC-YT) as well as the fungi *A. flavus*, *P. chrysogenum*, *F. moniliforme* ATCC 38932 and *Rhizopus* sp.

The methanolic extract of frozen juniper berry showed significant antimicrobial activity against *B. cereus* NCTC 11145, *M. luteus* 2YC-YT and *E. coli* ATCC 25922. The inhibitory effect on *B. subtilis* ATCC 6633, *B. amyloliquefaciens* 4BCL, *S. aureus* ATCC 25923, *L. monocytogenes* 8632, *E. faecalis* ATCC 19433, *S. enteritidis* ATCC 13076*, P. aeruginosa* ATCC 9027 and the fungus *A. flavus* was moderate. The antimicrobial activity against *K. pneumoniae* ATCC 13883, the yeast *S. cerevisiae* ATCC 9763, and the fungi *Rhizopus* sp. and *Mucor* sp. was low. Similar to cranberry, the extract of dried juniper berry showed a stronger inhibitory effect on some microorganisms in comparison with that of frozen fruit. The antimicrobial activity of the extract of dried juniper berry against *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145 and *M. luteus* 2YC-YT was significant, whereas against *B. amyloliquefaciens* 4BCL, *S. aureus* ATCC 25923, *L. monocytogenes* 8632, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 9027 was moderate. The inhibitory activity against *E. faecalis* ATCC 19433, *S. enteritidis* ATCC 13076, *S. typhimurium* 1672, *K. pneumoniae* ATCC 13883, *P. vulgaris* ATCC 6380, the yeast *S. cerevisiae* ATCC 9763, and the fungi *P. chrysogenum*, *F. moniliforme* ATCC 38932 and *Rhizopus* sp. was low. Methanol used as a solvent and a control did not show inhibitory effect on the test microorganisms (data not presented).

Rovna et al. [53] determined that ethanolic extracts of *R. canina* from three regions of Slovakia exerted the highest antimicrobial activity against *E. coli* and *Klebsiella oxytoca*. Eldaw and Çiftci [54] found the highest antibacterial potential of rosehip from 10 different regions of Samsun, Turkey against *E. faecalis*, while the lowest antibacterial activity was observed against *E. faecium*. Montazeri et al. [55] investigated the antibacterial activity of different rosehip extracts from Iran. The authors found that methanolic extracts showed strong antimicrobial activity against *S. aureus, E. coli, B. subtilis* and *C. albicans*. Kumarasamy et al. [56] stated the effectiveness of methanolic extract of *R. canina* seeds on the growth of *Lactobacillus plantarum*, *P. mirabilis* and *Staphylococcus epidermidis.* Ivanova et al. [1] established that methanolic extracts from wild and cultivated rosehip showed significant inhibitory activity against *M. luteus* 2YC-YT, moderate inhibitory effect on *B. subtilis* ATCC 6633, *L. monocytogenes* NBIMCC 8632, *E. coli* ATCC 25922, *P. vulgaris* ATCC 6380, *P. aeruginosa* ATCC 9027, and low inhibitory effect on *B. amyloliquefaciens* 4BCL-YT, *S. aureus* ATCC 25923, *E. faecalis* ATCC 19433, *S. enteritidis* ATCC 13076, *S. typhimurium* NBIMCC 1672 and *K. pneumoniae* ATCC 13883. Both rosehip extracts did not show antifungal activity, except against *Rhizopus* sp. and *F. moniliforme* ATCC 38932 (low inhibitory activity).

Krawitz et al. [57] stated that the extract obtained from elderberry exhibited antimicrobial activity against some human respiratory pathogens, such as *Streptococcus pyogenes*, group C and G *Streptococci* and *Branhamella catarrhalis*. The extract also demonstrated an inhibitory effect on the human influenza A and B viruses. The inhibitory effect of an elderberry extract on the human influenza virus was confirmed by Torabian et al. [58]. Haş et al. [45] proved that the most sensitive to the inhibitory activity of a methanolic elderberry extract were *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 (MIC = 1.95 mg/ml). Przybylska-Balcerek et al. [59] determined that the most sensitive to the extracts of elderberry were *M. luteus* PCM 525, *P. mirabilis* PCM 1361, *Pseudomonas fragii* PCM 1856 and *E. coli* PCM 2793 (MIC = 0.05 %).

Laslo and Köbölkuti [60] studied the antimicrobial properties of cranberry (*V. vitis-idaea* L.) from five areas in the Eastern Carpathians and stated that the extracts possessed inhibitory effect on Gram-negative and Gram-positive bacteria. The diameters of the inhibition zones varied as follows: 22.85-36.42 mm (*P. aeruginosa*), 21.58-30.33 mm (*Pseudomonas fluorescens*), 12.33-17.66 mm (*E. coli*), 9.84-16.37 mm (*Serratia marcescens*), 16.6-26.68 (*S. aureus*), 15.34-20.51 mm (*B. cereus*), 11.41-18.86 mm (*L. monocytogenes* S2), 12.41-18.25 mm (*B. subtilis*) and 9.4-14.84 mm (*L. monocytogenes* S1). In addition to its antimicrobial potential, Nikolaeva-Glomb [61] established that the total extract of cranberry (lingonberry) showed *in vitro* inhibitory activity against human coxsackievirus B1 (CV-B1) and influenza A virus, while the anthocyanin fraction effectively inhibited the replication of influenza virus A/Aichi/2/68(H3N2).

Miceli et al. [62] found that methanolic extracts of *J. communis* var. *communis* and *J. communis* var. *saxatilis* berries showed inhibitory activity against Gram-positive bacteria (*S. aureus* ATCC 6538P, *Enterococcus hirae* V3, *B. subtilis* P3, *S. epidermidis* G1), but were not active against the Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 9021, *P. mirabilis* G4) and yeasts (*C. albicans* ATCC10231, *C. parapsilosis* P7) tested in the study. The MIC values of both extracts ranged between 156.25 and 1250 µg/ml. The negative control (methanol) did not inhibit the growth of the tested strains, which was in agreement with our results. Fierascu et al. [20] investigated a hydroalcoholic extract of a wild growing *J. communis* L. from Romania and found that it exhibited high antifungal activity against *A. niger* and *Penicillium hirsutum* with diameter of the inhibition zones of 21.6 mm and 17.2 mm, respectively.

3.4 In vitro anti-inflammatory activity

The protein denaturation is an indicator for the presence of an inflammatory process. During this process, the secondary and tertiary structures of proteins are destroyed and a disturbance in their biological functions is observed. The ability of a certain substance to reduce the inflammation is associated with an anti-inflammatory effect [63]. Some anti-inflammatory drugs have been shown to inhibit the thermally induced denaturation of protein by a dose-dependent manner [64].

The *in vitro* anti-inflammatory activity was evaluated by assessing the degree of resistance of albumin molecule to thermally induced denaturation in the presence of plant extracts. The results were presented as percentage of inhibition of albumin denaturation as well as a half of maximal inhibitory concentration, or IC_{50} (Fig. 2 and Fig. 3). The juniper berry extract exhibited the highest anti-inflammatory effect, expressed as inhibition of the thermally induced albumin denaturation (79.68 %) and as well as the lowest IC_{50} value (6.28 mg/ml), followed by the extracts of elderberry (68.65 % and IC_{50} = 7.28 mg/ml), rosehip (34.74 % and IC_{50} = 14.39 mg/ml) and cranberry (23.41 % and IC_{50} = 21.37 mg/ml). As seen from the obtained results, the extracts of juniper berry and elderberry at concentration of 10 mg/ml exhibited higher values of albumin protection than those of the commercial anti-inflammatory drugs (58.44 % for aspirin and 57.34 % for prednisolone) used as controls at the same concentration. Furthermore, the values of IC_{50} of juniper berry and elderberry extracts were lower as compared to those of the controls (8.56 mg/ml for aspirin and 8.72 mg/ml for prednisolone).

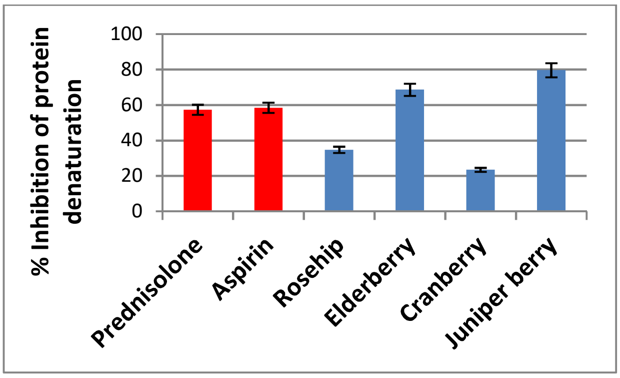

Fig. 2. Anti-inflammatory activity of the fruit extracts of four medicinal plants and the controls (10 mg/ml) expressed as inhibition of the albumin denaturation (%) (dried fruit material).

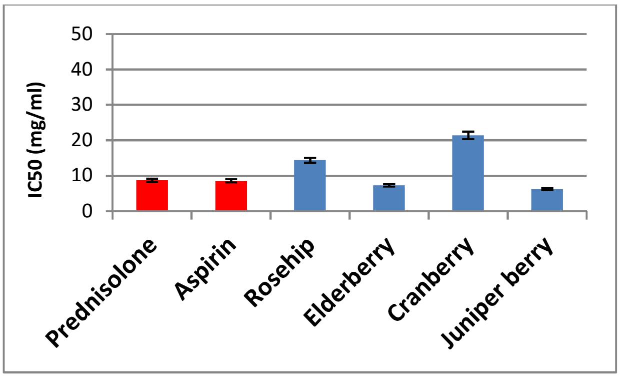

Fig. 3. Anti-inflammatory activity of the fruit extracts of four medicinal plants and the controls expressed as IC_{50} (dried fruit material).

At present, the scientific information about *in vitro* anti-inflammatory activity of the medicinal plants included in the present study is very limited.

A study conducted by Djellouli et al. [65] on the *in vitro* anti-inflammatory activity of methanolic and aqueous extracts from aerial parts of *Juniperus oxycedrus* from

Algeria, revealed high inhibition of albumin denaturation in a dose-dependent manner. The highest inhibition was observed at the highest concentration (1000 µg/ml), at which the methanolic extract showed value of 81.95 % vs. 32.48 % of the aqueous extract. These values were lower than that of the standard anti-inflammatory drug diclofenac sodium (91.51 %).

Widely used in medicine, non-steroidal (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs) can suppress inflammation process quickly and effectively, however they have many negative health effects associated with high doses or prolonged use. NSAIDs can cause stomach irritation, gastric ulcers and lower gastrointestinal tract disorders [64, 66]. SAIDs suppress the immune system, thus increasing the risk of infections as well as adrenal insufficiency, peptic ulcer disease, glaucoma, cataracts, psychosis, depression, hyperglycaemia, diabetes and osteoporosis [67]. Based on the results, we can conclude that the great anti-inflammatory potential of juniper berry and elderberry can find successful practical application in designing new medical products as an alternative of the conventional anti-inflammatory drugs.

4 Conclusion

In recent decades, the herbs become increasingly popular as attractive pharmaceutical, cosmetic and functional food ingredients. The results obtained from our study revealed that rosehip, black elder, cranberry and juniper fruits from Dospat region, Western Rhodopes, Bulgaria, represent high quality products, some of which possess great antioxidant, antimicrobial and anti-inflammatory potential. To the best of our knowledge, for the first time some physicochemical properties of these herbs were determined. For the first time the inhibition of protein denaturation assay was applied to determine the *in vitro* anti-inflammatory activity of these herbs. The juniper berry and elderberry extracts exhibited significant *in vitro* anti-inflammatory potential, which was higher compared to that of the conventional anti-inflammatory drugs prednisolone and acetylsalicylic acid (Aspirin). This can find future application in the development of novel pharmaceutical and cosmetic formulations as well as functional food products.

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