

Assessment of the Antioxidant Effect of Ethanolic Extracts Obtained from *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench Collected from the Eastern Part of Romania

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Numerous plants are currently used for both their curative and preventive effects in a wide range of pathologies. They possess multiple beneficial effects, including the well-known antioxidant capacity. *Agrimonia eupatoria* L. and *Filipendula* spp., from the Rosaceae family have emerged as a potential source of phenolic constituents, showing antioxidant activities. The aim of this study was to obtain ethanolic extracts from *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench. collected from the West part of Romania and to determine their antioxidant capacity. Two DPPH assays were used to determine the antioxidant activity of the extracts, ethanolic solution of 1 and 0.1 mM DPPH. Our data indicated that all the extracts, regardless of the place of harvest possess significant antioxidant activity. The most potent activity was recorded for the extracts prepared from *Agrimonia eupatoria* L. collected from Bazia^o – Locva Mountains in mixture with ethanolic solution of 0.1 mM DPPH, followed by *Filipendula ulmaria* (L.) Maxim, using the same concentration of ethanolic solution of standard antioxidant.

Key words: antioxidant capacity, DPPH, *Agrimonia eupatoria*, *Filipendula* spp., Rosaceae

Plants, vegetal products or secondary metabolites are widely used nowadays due to their beneficial effects in human health for both prevention and treatment in a wide variety of diseases [1]. An increased number of studies involving medicinal plants have been conducted due to their potential beneficial effects in severe pathologies, including cancer [2], cardiovascular diseases, diabetes, pulmonary and brain diseases [3, 4]. One of the mechanisms responsible for the protective effects is considered to be plants capacity to reduce oxidative stress [3]. The oxidative stress is considered to be an imbalance in the redox homeostasis between the excessive production of reactive oxygen species (ROS) and an impairment in the antioxidant capacity [5]. This can occur in various diseases like cancer, diabetes, vascular and brain dysfunction, aging, etc. [6]. The antioxidant effect can be due to single molecules within the extract or to the synergy of different phytochemicals [3].

Agrimonia eupatoria L. (agrimony) from Rosaceae family has been studied for its pharmacological properties, like hypoglycemic and hepatoprotective effects [6]. In recent years, it has been indicated that agrimony contains polyphenols responsible for the antioxidant capacity of the plant extracts. Furthermore, it has been stipulated that agrimony tea consumption induced antioxidant activity, increased the high density lipoprotein (HDL) cholesterol and reduced inflammation [7]. Granica *et al.* conducted HPLC studies and reported that *Agrimonia eupatoria* L. extracts contain multiple polyphenols including phenolic acids, flavonoids, ellagitannin and flavan-3-ol derivatives [8]. Furthermore, it was recently published that agrimony water infusion and a polyphenol-enriched fraction elicited antioxidant, analgesic and anti-inflammatory activities [9].

Filipendula spp, also belonging to the Rosaceae family, were used in the traditional medicine as anti-inflammatory

plants [10]. Recent studies indicated that *Filipendula* possesses significant antioxidant activity [10], antimicrobial [11], anti-inflammatory and gastroprotective [12] effects; moreover they were rated as non-mutagenic species under *in vitro* conditions [10]. Olennikov and coworkers indicated that *Filipendula ulmaria* (L.) Maxim. (meadowsweet) and *F. vulgaris* Moench. (dropwort) represent the most studied species from this genus and they contain various components, such as flavonoids, tannins, essential oils, salicylic acid derivatives etc. [13].

The present study was purposed to evaluate the antioxidant effects of ethanolic extracts from three plants belonging to the Rosaceae family, namely *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench, collected from the West part of Romania, using two concentration of the standard antioxidant, 1 and 0.1 mM.

Experimental part

Materials and method

Chemicals and reagents

Ethanol 96% (v/v) was purchased from Chemical Company SA, Iasi, Romania; 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Batch No: # STBF5255V), was acquired from Sigma Aldrich Company. The ascorbic acid was acquired from Lach-Ner Company (Czech Republic).

Harvesting of the plants

Plants were harvested at the beginning of July (3-10.07.2016) from different cities from Caras-Severin region. Healthy plants were harvested during the flowering period at noon. Fresh material was processed the same day. The collected plants were identified and deposited in the Herbarium Department of Medicinal Plants, Banat's

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University of Agricultural Science and Veterinary Medicine Timisoara. Each plants was assigned a voucher specimen.

Agrimonia eupatoria L. (*herba*) was collected from four places, namely Carasova (Anina Mountains) – sample A1 (vocher specimen code VSNH.BUASTM -118); Comorăte (Barzava Field) - sample A2 (vocher specimen code VSNH.BUASTM - 119); Bazias (Locva Mountains) – sample A3 (vocher specimen code VSNH.BUASTM - 120); and Prilipet (Almaj Mountains)- sample A4 (vocher specimen code VSNH.BUASTM -121).

Filipendula ulmaria (L.) Maxim. (*herba*) was collected from Lăpusnicel (Almaj Mountains) - sample F1 (vocher specimen code VSNH.BUASTM - 122), and *Filipendula vulgaris* Moench. (*herba*) was harvested from Petnic (Almaj Mountains) - sample F2 (vocher specimen code VSNH.BUASTM - 123).

Extraction method

The vegetative organs used for the extraction were weighed and minced. Then, were placed in dark glass bottles over which the hydroalcoholic solution was added. For 5 g of plant material, 50 mL of ethanolic solution 45% was used. The extracts obtained were left in the dark for 14 days, after which they were decanted through filter paper, passed into glass containers and stored cold until further uses. The schematic protocol is described in figure 1.

Antioxidant activity (AOA) assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical frequently used to determine the antioxidant activity of various types of extracts, described for the first time by Blois (1958) [14]. The experimental method is based on its capacity to reduce from the dark violet DPPH in the presence of an antioxidant, to a pale yellow compound. For the evaluation of the antioxidant activity (AOA), two alcoholic solutions of 1 and 0.1 mmol·L⁻¹ DPPH in ethanol 96% (v/v) were prepared and stored at 4°C, in the dark (DPPH solutions are used as a standard antioxidant). The data obtained was compared with an etalon solution,

consisting of an alcoholic solution of ascorbic acid (2 mmol·L⁻¹) in ethanol 96% (v/v).

According to the Manzoco et al. (1998) [15] slightly modified method, in a 4 mL cuvette was added: 0.5 mL of each sample, 0.5 mL DPPH solution 1 mM and 2 mL solvent (the solvent in which the extracts were dissolved - ethanol 45%). Using the same method, it was analyzed also the etalon solution of 2 mM ascorbic acid in ethanol 96%. The absorbance of the mixture was measured at 516 nm, for 1200 s continuous, using a T80 UV/Vis Spectrophotometer (PG Instruments Ltd).

According to Wu et al. (2003) [16], in a 4 mL cuvette was added 0.1 mL of each sample including the ascorbic acid alcoholic solution (2 mM) and 3 mL of 0.1 mM DPPH ethanolic solution. The absorbance of each sample was measured, continuously, at 516 nm, for 1200 s, using the same type of spectrophotometer.

The antioxidant activity (%AOA) regarding the two procedures used, has been calculated using the following equation:

$$AOA(\%) = \frac{A_i - A_f}{A_i} \cdot 100 \quad (1)$$

where:

AOA - antioxidant activity of the analysed samples (5);

A_i - the absorbance of the standard antioxidant (DPPH 1/01 mM);

A_f - the absorbance of the mixture (sample / ascorbic acid solution + DPPH 1 / 01 mm) after reaction occurs.

Statistical analysis

The analyses were done in triplicate. The data from the present study were analyzed using Origin 8 (OriginLab – Data analysis and Graphing Software).

Results and discussions

a) Evaluation of antioxidant activity using DPPH 1 mM

In figure 2 it is represented the antioxidant activities (AOA) of the alcoholic extracts obtained from *Agrimonia eupatoria* L. in time compared to the antioxidant activity of

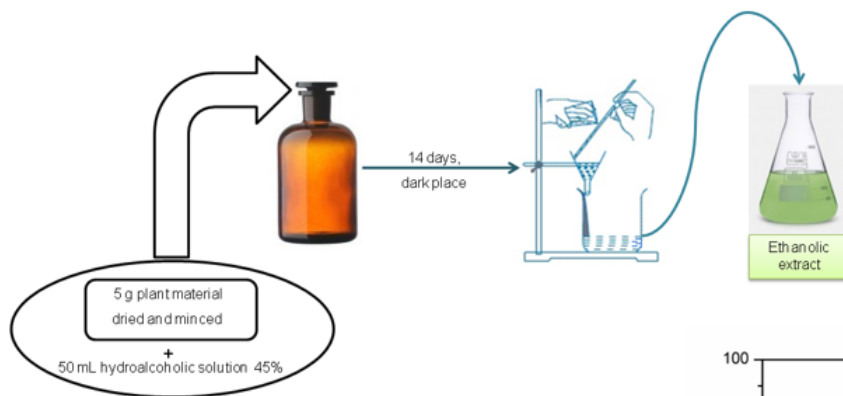
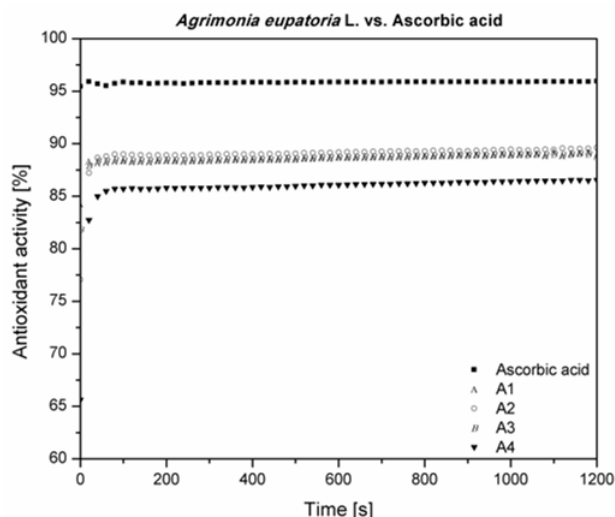


Fig. 1. The schematic protocol of the ethanolic extracts.

Fig. 2. AOA of ethanolic extracts obtained from *Agrimonia eupatoria* L. versus ascorbic acid, with DPPH 1 mM, in time



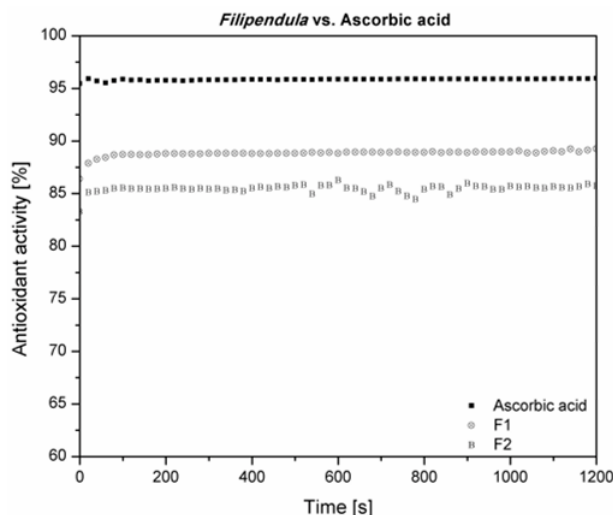


Fig. 3. AOA of the ethanolic extracts obtained from *Filipendula* species, versus ascorbic acid, with DPPH 1 mM, in time

Table 1

THE AOA OF THE FOUR ALCOHOLIC EXTRACTS OF *AGRIMONIA EUPATORIA* L. AS COMPARED WITH THE AOA OF ETHANOLIC SOLUTION OF ASCORBIC ACID, AT THE FIRST CONTACT BETWEEN THE EXTRACT AND DPPH 1 mM AND AT THE FINAL MOMENT OF THE REACTION, RESPECTIVELY

Procedure	Sample	Antioxidant activity [%]	
		Initial moment (time 0)	Final moment (after 1200 secunde)
0.5 mL ethanolic extract + 0.5 mL DPPH 1 mM + 2 mL EtOH 45%	A1	86.13±2.57	89.09±0.37
	A2	80.91±5.43	89.53±0.12
	A3	84.39±3.59	89.07±0.03
	A4	71.96±8.97	86.54±0.03
0.5 mL ascorbic acid + 0.5 mL DPPH 1 mM + 2 mL EtOH 96%	Ascorbic acid	95.63±0.27	95.93±0.03

*The results are expressed as means±SD (n=3)

ascorbic acid (AOC) used as reference. Tested extracts, together with the etalon ascorbic acid, were recorded continuously for 20 min in order to evaluate the time needed for the consumption of the standard antioxidant to determine the speed at which DPPH was consumed by the antioxidants present in the alcoholic extracts. It can be seen from the chart that all the extracts show AOA, the highest value being recorded by the alcoholic extract obtained from the plant collected from Comoraste (Barzava Field) (sample A2 = 89.6% vs. Ascorbic acid = 95.9%).

Furthermore, the samples collected from Carasova (Anina Mountains) - sample A1; Comorâte (Barzava Field) - sample A2 and Bazias (Locva Mountains) - sample A3 have approximately the same antioxidant value when reacting with DPPH radical. On the other hand, weaker

antioxidant activity was observed in the case of the sample collected from Prilipet (Almaj Mountains) - sample A4. Regarding the time when DPPH was consumed by the antioxidants contained in the extracts, this process occurred very fast, more exactly in the first 50 s of analysis, after which the reaction reached equilibrium.

In table 1 are presented the antioxidant values of the alcoholic extracts, including the antioxidant value of ascorbic acid, at the initial moment (at the first contact of the extract with DPPH, $t = 0$ s), and when the reaction was finished (after 1200 s of analysis), respectively.

Figure 3 presents the AOA of the extracts obtained from two species of *Filipendula*, namely: *Filipendula ulmaria* L. Maxim. collected from Lapu'nicel (Almaj Mountains) -

Table 2

THE AOA OF THE TWO ALCOHOLIC EXTRACTS OF *FILIPENDULA* SPECIES AS COMPARED WITH THE AOA OF ETHANOLIC SOLUTION OF ASCORBIC ACID, AT THE FIRST CONTACT BETWEEN THE EXTRACT AND DPPH 1 MM AND AT THE FINAL MOMENT OF THE REACTION, RESPECTIVELY

Procedure	Sample	Antioxidant activity [%]	
		Initial moment (time 0)	Final moment (after 1200 secunde)
0.5 mL ethanolic extract + 0.5 mL DPPH 1 mM + 2 mL EtOH 45%	F1	86.98±0.74	89.34±0.03
	F2	84.19±1.17	85.80±0.02
0.5 mL ascorbic acid + 0.5 mL DPPH 1 mM + 2 mL EtOH 96%	Ascorbic acid	95.63±0.27	95.93±0.03

*The results are expressed as means±SD (n=3)

sample F1 and *Filipendula vulgaris* Moench. (herba) harvested from Petnic (Almaj Mountains)-sample F2.

It can be observed that even in the case of *Filipendula* species, both extracts shown antioxidant activity compared with ascorbic acid. The *Filipendula ulmaria* species (sample F1), harvested from Lapusnicel-Almaj Mountains, showed an increased antioxidant activity in comparison with the AOA of *Filipendula vulgaris* species (sample F2), harvested also from the Almăj Mountains but from another village (Petnic). From the chart it can be observed the fact that the *Filipendula ulmaria* extract reacted with DPPH and consumed it in the first 100 s of analysis, while the *Filipendula vulgaris* extract reacted and consumed the standard antioxidant in the first 10 s of analysis. Consuming the standard antioxidant over the time, leads to an increased AOA due to the high content of biologically active compounds in the extract.

Initially, all the extracts had lower AOA (before the reaction with the standard antioxidant had started, but at the final moment (after 1200 s, at the end of the reaction), all the extracts presented an increased antioxidant activity. This fact could be better observed in table 2.

b) Evaluation of antioxidant activity using DPPH 0.1 mM

In order to verify if the high concentration of standard antioxidant (1 mM) could influence the values of AOA of the tested extracts, an ethanolic solution of 0.1 mM DPPH was prepared, and all the extracts from the tested species (*Agrimonia eupatoria* L. and *Filipendula*) were again analyzed, following the procedure developed by Wu and collaborators [16].

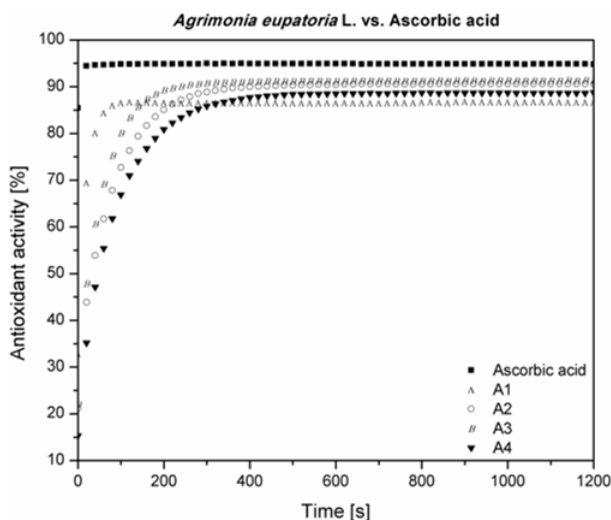


Fig. 4. AOA of ethanolic extracts obtained from *Agrimonia eupatoria* L. versus ascorbic acid, react with DPPH 0.1 mM in time

Procedure	Sample	Antioxidant activity [%]	
		Initial moment (time 0)	Final moment (after 1200 secunde)
0.1 mL ethanolic extract + 3 mL DPPH 0.1 mM	A1	44.71±16.66	86.72±0.05
	A2	27.69±9.29	90.65±0.06
	A3	29.81±10.94	91.37±0.06
	A4	21.17±8.23	88.66±0.06
0.1 mL ascorbic acid + 3 mL DPPH 0.1 mM	Ascorbic acid	89.90±6.33	94.81±0.06

*The results are expressed as means±SD (n=3)

Figure 4 present the AOA of the four extracts obtained from *Agrimonia eupatoria* L., when the extracts were mixed with 0.1 mM DPPH ethanolic solution.

Compared to the extracts evaluated with 1 mM DPPH ethanolic solution, these samples showed increased values of AOA. When the ethanolic solution of 0.1 mM DPPH was used, the extract with the highest value of AOA was the sample A3, from the plant collected from Bazias (Locva Mountains), followed by the sample A2 (extract prepared with plant collected from Comoraste - Barzava Field), then by the sample A4 (extract prepared with plant collected from Prilipet - Almaj Mountains) and finally by the sample A1 (extract prepared with plant collected from Carasova - Anina Mountains).

The AOA value obtained in the case of ethanolic extract A3, was very close to the AOA value of ascorbic acid (A3 = 91.33% vs. Ascorbic acid = 94.76%). We believe that the value obtained for the sample A3, with a difference of only 3% against the etalon is due perhaps to the area where the plant was harvested, that is part of a geographically protected area and the pollution level is very low or even missing.

It is also thought that the AOA values obtained for the sample A1 are the lowest due to the place where the plant was harvested, namely, Carasova - Anina Mountains. It is known the fact that formerly, Anina was considered the mainly mining town from the Western part of Romania. In the communism period, the construction of an electric power station has been attempted, but with the first overflowing of hot ashes resulting from the heating, the underground of the city has caught fire, still burning in the present. It is very likely that due to the pollution in the area, the AOA value was lower. The low AOA value can also be seen from the reaction speed between the 0.1 mM DPPH ethanolic solution and the antioxidants present in the extract, they consume the standard antioxidant in the first 100 s.

Regarding the reaction speed between the 0.1 mM DPPH ethanolic solution in mixture with the antioxidants present in the A3, A2 and A4 extracts, these ones consumed the DPPH after 400 s of analysis, and then the reactions reached equilibrium.

The values of AOA regarding the four ethanolic extracts compared with the AOA of ascorbic acid ethanolic solution of 2 mM, are presented in table 3.

Towards the extracts analyzed with 1 mM DPPH ethanolic solution, the same extracts analyzed with 0.1 mM DPPH displayed, at the first contact of the extract with the standard antioxidant (initial moment), values much lower of AOA.

We can conclude that, regarding the *Agrimonia eupatoria* L. extracts, the AOA values are inversely

Table 3
THE AOA OF THE FOUR ALCOHOLIC EXTRACTS OF *AGRIMONIA EUPATORIA* L. AS COMPARED WITH THE AOA OF ETHANOLIC SOLUTION OF ASCORBIC ACID, AT THE FIRST CONTACT BETWEEN THE EXTRACT AND DPPH 0.1 mM AND AT THE FINAL MOMENT OF THE REACTION, RESPECTIVELY

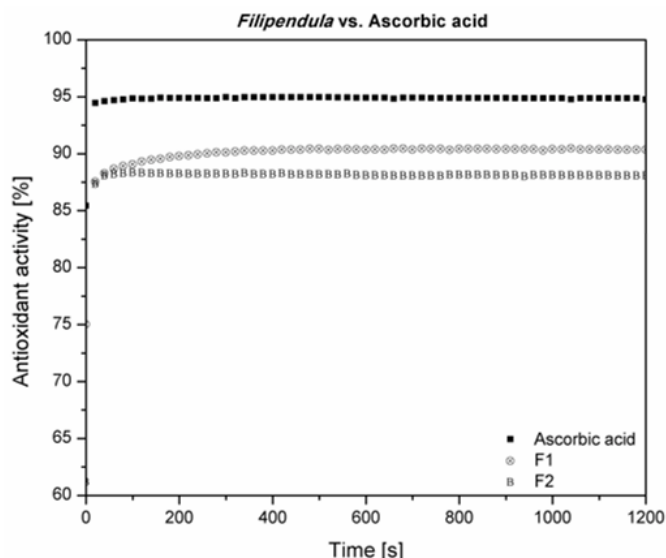


Fig. 5. AOA of the ethanolic extracts obtained from *Filipendula* species, versus ascorbic acid, with DPPH 0.1 mM, in time

proportional with the used concentration of the standard antioxidant. The higher the concentration of the standard antioxidant, the lower the AOA values of the prepared ethanolic extracts are.

In figure 5 are presented the AOA of the two species of *Filipendula* compared with the AOA of ascorbic acid 2 mM.

Concerning the *Filipendula* species, the concentration of DPPH did not influence significantly the final value of the AOA of the two extracts. Between the *Filipendula ulmaria* L. and *Filipendula vulgaris* Moench, there is a slight difference of about 2%. Even when the ethanolic solution of 0.1 mM DPPH was used, the highest value of AOA was shown in the case of the extract prepared from *Filipendula ulmaria* L., harvested from Lapusnicel - Almaj Mountains.

We can conclude that in this case, the extracts obtained from *Filipendula* species do not depend on the concentration of the standard antioxidant used.

In table 4, are presented the values of the AOA regarding the *Filipendula* species extracts versus AOA values of the ethanolic solution of ascorbic acid, at the initial moment and at the final moment respectively.

Previous studies have shown that *Agrimonia eupatoria* L. and *Filipendula* spp. possess a significant antioxidant capacity [6, 10, 13]. Kuczmanna *et al.* indicated that a water infusion obtained from *Agrimonia eupatoria* L. had the ability to scavenge free radicals, inducing an antioxidant effect. The authors indicated that this can be due to the fact that agrimony extract contains numerous phenolic agents including apigenin, kaempferol, quercetin and also proanthocyanidins, the decelate concentration of

polyphenols in the extract being 8% [6]. In another study, it has been shown that agrimony aqueous extract has significant amounts of polyphenols, flavonoids and tannins, compounds responsible for the antioxidant activity [8].

Several agrimony extracts (water, diethyl ether, acetone, and ethanol extracts) have been characterized by Muruzovic and collab., regarding the total phenolic content, tannins, flavonoids and proanthocyanidins; they also determined the antioxidant activity of the aforementioned extracts [17]. It has been established that the highest total phenolic content was obtained in the case of the acetone extract and also the strongest antioxidant activity, determined by a DPPH methanolic solution, was reported for the acetone extract (97.13 ± 0.26 versus 97.18 for ascorbic acid used as reference substance). Similar results were obtained for the aqueous and ethanol extracts (93.95 ± 0.27 - aqueous extract and 94.88 ± 0.14 - ethanol extract) [17]. Our results are in concordance with these results, since we obtained an antioxidant activity above 85% for all the agrimony ethanol extracts, regardless of the concentration used for DPPH ethanol solution.

Concerning *Filipendula* species, Olennikov *et al.* investigated the antioxidant activity of four species (*F. camschatica*, *F. denudata*, *F. stepposa* and *F. ulmaria*) prepared as decoctions, using different assays (DPPH, ABTS, bromine free radicals scavenging and carotene bleaching assay) [13]. They established that *F. stepposa* decoction had the most significant antioxidant activity in all the tests, followed by *F. denudata*, *F. ulmaria* and *F. camschatica*, respectively.

In a study performed on methanolic extracts of *Filipendula ulmaria* (L.) Maxim. collected from Serbia, the authors proved that the extracts possess antioxidant properties, an effect that can be correlated to the high content in phenolic acids and flavonoids present in the aerial parts of the plant [11]. According to Maksimovič *et al.* the methanolic extract from *Filipendula hexapetala* (*F. vulgaris*) harvested from Serbia elicited a significant antioxidant activity (assessed by FRAP, DPPH and lipid peroxidation assay) [18]. Pukalskiene and coworkers discovered that *Filipendula vulgaris* methanol and acetone extracts have a high amount of phenolic structures and are efficient against DPPH radicals [19]. Furthermore, a hydroalcoholic extract from *Filipendula vulgaris* collected from Siberia elicited a pronounced antioxidant activity (80 ± 1.0 %) [20].

The ethanolic extracts of the two species of *Filipendula* tested in this study provoked a significant antioxidant activity, ranging from approximately 85% to 90%, the most active being the extract from *Filipendula ulmaria* (L.) Maxim tested with DPPH 0.1 mM ethanolic solution.

Table 4
THE AOA OF THE TWO ALCOHOLIC EXTRACTS OF *FILIPENDULA* SPECIES AS COMPARED WITH THE AOA OF ETHANOLIC SOLUTION OF ASCORBIC ACID, AT THE FIRST CONTACT BETWEEN THE EXTRACT AND DPPH 0.1 mM AND AT THE FINAL MOMENT OF THE REACTION, RESPECTIVELY

Procedure	Sample	Antioxidant activity [%]	
		Initial moment (time 0)	Final moment (after 1200 secunde)
0.1 mL ethanolic extract + 3 mL DPPH 0.1 mM	F1	80.93 ± 8.26	90.42 ± 0.05
	F2	72.73 ± 16.18	88.17 ± 0.04
0.1 mL ascorbic acid + 3 mL DPPH 0.1 mM	Ascorbic acid	89.90 ± 6.33	94.81 ± 0.06

*The results are expressed as means \pm SD (n=3)

Conclusions

The ethanolic extracts obtained from *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench. collected from Caras-Severin region, induced a significant antioxidant activity, the results being close to the value of ascorbic acid, used as standard. Our results indicate that the highest antioxidant activity was recorded for *Agrimonia eupatoria* L. harvested from Băzia⁰-Locva Mountains and for *Filipendula ulmaria* (L.) Maxim. harvested from Almăj Mountains, for both when the ethanolic solution of 0.1 mM DPPH was used.

The study shows that in the case of ethanolic extracts prepared from *Agrimonia eupatoria* L. the concentration of the standard antioxidant is very important, the values of the AOA obtained depending on it.

The results of the present work corroborate the traditional use of *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench. respectively, as antioxidants, and suggest that the species from West Romania possess high antioxidant capacity due to the polyphenols contained by the plants.

In the last years in a great number of papers was studied the antioxidant effect of medicinal plants collected from Romania [21].

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