Equisetum arvense L., field horsetail is herbaceous perennial fern. It is widely used in the world, because it has many health benefits. Steril stems are used as a treatment of diseases such as inflammation, anemia, diabetes, ulcers, cancer, convulsions, anxiety and depressive disorders. In this study we investigated the phenolic compounds and antioxidant capacities of free and bound phenolics from steril stems of Equisetum arvense L. We analyzed the composition of samples in phenolic compounds by HPLC method. Antioxidant capacity of the extracts was evaluated by the following methods: Cuprac assay, DPPH method, FRAP method. Predominant flavonoids identified in these analyses were epicatechin, quercetin, catechin while the major phenolic acids were vanillic acid, caffeic acid, ferulic acid and p-coumaric acid. The results showed that extracts of Equisetum arvense L. are rich sources of phenolic compounds.

**Keywords:** Equisetum arvense L., antioxidant activity, phenolic acids, flavonoids

Phenolic compounds are secondary metabolites of plants. These are involved in the defense against ultraviolet radiation and pathogen attacks [1, 2]. Several thousands of natural polyphenols have been identified in plants. Phenolic compounds are classified in two groups: flavonoids and non-flavonoids.

Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids. Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids. Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids. Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids. Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids. Flavonoids are benzopyran derivatives having a phenyl radical substituted in the position 2 [3]. In plants they are flavonoids and non-flavonoids.

Flavonoids and non-flavonoids are also major nutritional compounds in foods and beverages, that have been found to have antioxidant activities and other health-promoting properties [6-8]. Flavonoids are synthesized via the phenylpropanoid pathway, which is a ubiquitous and well-described plant secondary metabolite pathway [9]. The core flavonoid structure consists 15 carbon atoms with a common structure of diphenylpropanes, two aromatic rings with 6 carbon atoms interconnected by a heterocyclic ring that contains 3 carbon atoms [10, 11].

The group of non-flavonoids includes phenols, phenolic acids, coumarins, etc. Phenolic acids show a broad spectrum of pharmacological activity; they have antioxidant, antimutagenic, antitumor and anticarcinogenic properties [12-15]. They have also been found to possess antiviral and antibacterial properties [16].

Epidemiological data suggests that the consumption of plant-derived antioxidants such as flavonoids and phenolic acids prevent various diseases [17, 18]. However it accentuates the study of plants with high level of flavonoids and phenolic acids.

Equisetum arvense L., field horsetail is herbaceous perennial fern. It is widely used in the world, because it has many health benefits. Steril stems are used for treatment of diseases such as anemia, inflammation, diabetes, ulcers, cancer, convulsions, anxiety and depressive disorders [19-21]. Phytochemical studies of Equisetum arvense L. have reported the occurrence of inorganic compounds (especially silica), caffeic acid derivatives, flavonoids, alkaloids, saponins [22-25].

**Experimental part**

In this study the steril stems of Equisetum arvense L were investigated. In our previous studies the most increased values of phenolic compounds and flavonoids were identified in the stems harvested in May, so the studies have focused on the specimens collected in this month.

A study was performed, in which, we followed the bioactive compounds, polyphenols content and total flavonoid content. The composition of phenolic compounds in our samples were analyzed by HPLC method.

Antioxidant capacity of the extracts was evaluated by the following methods: CUPRAC assay, DPPH method, FRAP method.

**Plant materials**

Steril stems of Equisetum arvense L., were used. They were collected from Bihor county in May 2015, dried at an average temperature of 40°C, for 96 h.

**Extract preparation**

Three different extraction methods were used to produce phenolic extracts from the plant using the steril stems.

**Preparation of the lyophilized extracts (A)**

Aliquots of steril stems (10 g) were extracted with 70% aqueous ethanol (100 mL) using a magnetic mixer for 20 min and sonicated for 5 min. The sample was centrifuged (10 min, 3000 g) and the combined extract was vacuum evaporated to dryness on a rotary evaporator. The dried extract was transferred to a Petri dish with 10 mL of water.
and frozen overnight (-25°C), the extract was lyophilized and the residue weighed and transferred to a sample vial.

**Preparation of the plant neutral (unhydrolisic) extracts (B)**
About 50 mg of lyophilized extract (dry extract, A) was weighed and dissolved in 2 mL of 90% methanol and 0.5% acetic acid mixture, after to add 2 mL of intern standard (naringenin 10 mmol). The sample was centrifuged and the combined supernatant evaporated on a rotary evaporator to dryness. The residue was dissolved in 1 mL DMSO and transferred to a test tube.

**Preparation of the plant acidic (hydrolysis) extracts (C)**
About 50 mg of lyophilized extract (dry extract, A) was weighed and dissolved in 4 mL of 25% methanol, after to add 2 mL of intern standard (naringenin 10mmol) and 1mL of HCl (6M) the sample was sonicated for 2 min. and mixed in a water bath at 85-90°C for 2 h. Then 2.5 mL of ethylacetate was added to each sample and this extract was vacuum evaporated to dryness on a rotary evaporator. The residue was dissolved in 1 mL DMSO and transferred to a test tube.

**Preparation of Phenolic Standards for RP-HPLC**
Pure phenol standards including 7 flavonoids (catechin hydrate, rutine trihydrate, naringenin, luteolin, quercetin dihydrate, epicatechin, myricetin) and 7 phenolic acids (gallic, cafè, syringic, synapin, vanillic, p-coumaric, ferulic,) were dissolved in mobile phase as 1 mg/mL (gallic, café, syringic, synapin, vanillic, p-coumaric, ferulic,) hydrate, rutine trihydrate, naringenin, luteolin, quercetine to a test tube.

The mixture was allowed to react for 3 min and 3 mL aqueous solution of 2% Na,CO3 was added. At the end of the 2 h incubation at the room temperature, the absorbance of each mixture was measured at 765 nm in Shimadzu UV-1700 Pharmaspec UV-Vis Spectro-photometer. The same procedure was also applied to the standard solutions of gallic acid, and a standard curve was obtained [29].

The total flavonoid content was determined using a previously described colorimetric method [30]. 1 mL sample (containing 0.1 mg/mL dry substance) was mixed with 4 mL water and inserted in a 10 mL volumetric flask. Firstly, 3 mL 5% NaNO2 solution where added, after 5 minutes 0.3 mL 10% AlCl3, and after 6 minutes 2 mL 1M NaOH. The flask was filled up to its calibration mark with distilled water. The solution was mixed and its absorbance was detected at 510 nm [31].

**The antioxidant capacity of sterl stems extracts**

**DPPH method**
Radical scavenging activity of plant extracts against stable 2,2-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) was determined by the slightly modified method of Brand-Williams et al., in 1995 [32]. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol 6x10-5 M was prepared fresh daily before UV measurements. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

\[
\%\text{Inhibition} = \left( \frac{(AB - AA)}{AB} \right) \times 100
\]

where AB = absorption of blank sample (t= 0 min), AA = absorption of test extract solution (t = 15 min) [32-34].

**FRAP method (ferric reducing antioxidant power)**
A simple spectrophotometric method that assesses the antioxidant power of the studied samples, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid pH. The stock solutions included: 300 mM acetate buffer; 270 mg FeCl3· 6 H₂O dissolved in 50 mL distilled water; 150 mg TPTZ and 150 µL HCl, dissolved in 50 mL distilled water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL FeCl3· 6 H₂O solution and 5 mL TPTZ solution. Trolox was used as a standard solution, the calibration curve was made for concentrations between 0 and 300 µM, having a correlation coefficient R² = 0.9956 and the regression equation (y = 0.0017x + 0.0848), where y represents absorbance detected at 595 nm. The results are expressed as µmol Trolox equivalents (TE)/100 µL extract [34-36].

**Cupric ions (Cu²⁺) reducing-CUPRAC assay**
In order to determine the cupric ions (Cu²⁺) reducing antioxidant capacity the method proposed by Karaman et al., in 2010, was used with slight modifications [37]. To this end, 0.25 mL CuCl2 solution (0.01M), 0.25 mL ethanolic neocuproine solution (7.5x10⁻³ M) and 0.25 mL CH₃COONH₄ buffer solution (1 M) were added to a test tube, followed by mixing with the plants extracts. Then, total volume was adjusted to 2 mL with distilled water, and thoroughly mixed. The tubes were stopped and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank 30 min later. Increased absorbance of the reaction mixture indicates increased reduction capability [37-39].
Results and discussions

Studies have shown that there are differences between the content of the bioactive substances in *E. arvense* L. and plants collected in different geographical areas [40, 41]. Samples used in this study, originating from the spontaneous flora of Bihor county, were carefully selected from unpolluted areas.

The amounts of total polyphenols and flavonoids found in the ethanolic extract of sterile stems are shown in Table 1. These results are similar to those obtained in 2014. Greater amounts of flavonoids and polyphenols were obtained in other studies [41, 42] but the material used came from cultures not from spontaneous flora.

A large number of papers confirm various biological effects of the *E. arvense* extracts, such as sedative and anticonvulsive, hepatoprotective, antioxidant, antibacterial and antifungal activity [43-45].

Antioxidant capacity of the extracts was evaluated by the following methods: Cuprac assay, DPPH method, FRAP method. All analyses were mean of triplicate measurements ± standard deviation.

Cuprac assay and FRAP method are spectrophotometric methods to determine cupric ions and ferric ions reducing antioxidant capacity. DPPH is the stable radical and is often used for assessing the antioxidant activity of natural products [46]. The results are shown in table 2. *E. arvense* L., extracts exhibits a significant antioxidant activity.

Kukric et al [47] was determined 50% DPPH. Compared to these results we have obtained a more pronounced antioxidant activity. The results show a high antioxidant capacity in comparison with other medicinal herbs studied recently [48].

Results of the analysis of phenolic compounds by HPLC are shown in figure 1, 2 and 3. Chromatograms of the *E. arvense* L., stems extracts recorded at 270, 310 and 360 nm as seen in figures 1, 2 and 3, to highlight a greater number of phenolic compounds.

Concentration of flavonoids and phenolic acids components in the sterile stems of *Equisetum arvense* L. are shown in table 3.

Predominant flavonoids identified in these analyses were epicatechin, quercetin, catechin while major phenolic acids were vanillic acid, caffeic acid, ferulic acid and p-coumaric acid, as seen in table 3.

Unhydrolysis and acid hydrolysis extracts were also prepared, because some phenolic compounds are bound to carbohydrates so it can highlight them only in acidic medium.

Between the flavonoids, epicatechin in unhydrolysis extracts is lower than in acid hydrolysis extracts. In case of catechin is inversely, but the quantities are much lower. Quercetin could highlights only in acid hydrolysis extracts [49]. Rutin founds in small quantities in neutral extracts, in acid hydrolysis extracts is transformed in quercetin. Epicatechin is classified in flavonols subgroup, but quercetin in flavonols subgroup, their antioxidant actions are well studied [18, 19, 50, 51].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total polyphenolic content (mg GAE/100DW)</th>
<th>Total flavonoid content (mg QE/100 DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Equisetum arvense</em> L. steril stems</td>
<td>82.63±0.06</td>
<td>71.23±4.33</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>FRAP (μmol Trolox equivalent/gDW) (TE)</th>
<th>DPPH %</th>
<th>Cuprac (μmol Trolox equivalent/g DW) (TE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Equisetum arvense</em> L. steril stems</td>
<td>8.160±0.78</td>
<td>87.30±0.039</td>
<td>49.2±0.104</td>
</tr>
</tbody>
</table>

Table 2

![Fig. 1. Chromatograms at λ = 270 nm of the unhydrolysis (neutral) (B) and acid hydrolysis extract (C)](image1)

![Fig. 2. Chromatograms at λ = 310 nm of the unhydrolysis (neutral) (B) and acid hydrolysis extract (C)](image2)
Between the non-flavonoids gallic acid is a trihydroxybenzoic acid, which is the most common type of phenolic acid in plants [52], but it is present in small quantities in field horsetail stem extracts. The other non-flavonoids, vanillic acid and caffeic acid are presents in the largest quantities, mainly in acid hydrolysis extracts. Vanillic acid is a dihydroxybenzoic acid, but caffeic acid, ferulic acid, P-coumarinic acid and syringic acid are hydroxycinnamic acids, of these caffeic acid is the most common in plants [52, 53]. Our results show that antioxidant compounds identified in field horsetail stem extracts in the largest quantity are conjugates of organic compounds, so that they can be used more effectively in cell metabolism than in the form of aglycones.

Conclusions

The results showed that extracts of Equisetum arvense L. are rich sources of phenolic compounds, flavonoids and phenolic acids, with antioxidant capacity and reducing power. This study has provided useful information for screening this plant as potential source of bioactive components with antioxidant properties that may be included in dietary supplements helpful in preventing different diseases.

References

18. SALVATORE, C., Inflammatory and allergy-drug targets, 9, no. 4, 2010, p. 263.
22. VEIT, M., Zeitschrift für Phytoresearch, 15, no. 6, 1994, p. 331.

Manuscript received: 16.12.2015