

The Comparative Study of *Equisetum pratense*, *E. sylvaticum*, *E. telmateia*: Accumulation of Silicon, Antioxidant and Antimicrobial Screening

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The objective of the current study is represented by the determination of silica and a phytochemical screening of phenolic derivatives of some Equisetum species. The antioxidant and antimicrobial activity for Equisetum pratense Ehrh., Equisetum sylvaticum L. and Equisetum telmateia Ehrh. (sin. Equisetum maximum Lam.) were also investigated. The concentration of silicon (Si) in plants was determined by the spectrophotometric method using previous treatment with NaOH 50% both for the stem and the nodal branches [1]. Results obtained varied from 95.12 to 162.10 SiO₂ mg/g dry plant which represents 4.44% to 7.58% Si/100g dry sample. Two types of total extracts were obtained using different solvents and were subjected to qualitative and quantitative chemical analysis considering total phenolic content [2]. The highest concentration of investigated compounds was found in the methanolic extract, E. sylvaticum, 196.5mg/g dry sample. Antioxidant activity was monitored spectrophotometrically and expressed in terms of IC₅₀ (μg/mL) [3]. Values gathered ranged from 261.7 to 429.5 μg/mL. The highest capacity to neutralized DPPH radicals was found in E. sylvaticum. In vitro antimicrobial activity was determined using difusimetric method [4]. Testing was performed on four microorganisms: three strains of bacteria and one species of fungi. Different effects were noticed against the bacteria, furthermore the methanol extract appeared to be most efficient. All extracts showed significant antimicrobial activity against Staphylococcus aureus (ATCC 25923) and Candida albicans (ATCC 90028) and weak to no activity against Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 25922).

Keywords: *Equisetum* species, silicon, phenols, antioxidant capacity, antimicrobial activity

One significant class of ferns is *Equisetopsida* which consists of three orders: *Protoarticulatae*, *Sphynophyllales*, and *Equisetales*. The *Equisetales* order contains only one family, *Equisetaceae* and one genus, *Equisetum* [5]. This family consists of approximately fifteen species and hybrids, spread worldwide from regions with an equatorial to those with a temperate or cold climate [6]. Nine of these species, commonly known as *horsetail*, are found in the Romanian flora [7].

These are perennial plants which have spontaneously developed and are frequently found in forests, wet meadows and swamps [8]. According to literature, these species possess a horizontal, ramified and articulate rhizome; in addition, at every node they possess a small whorl of small scaly leaves which is welded at the base. The sporangia are borne on the sporangiophores which are aggregated on a compact structure called sporangiferous spike. The latter may be present both on the vegetative and the specialized stem. The spores of this species are spherical, filled with tightly packed chloroplasts and four twisted elaters with a role in dissemination. Lastly, the gametophyte is present at the surface of the soil and bears a role in photosynthesis [9, 5-8].

The plant contains several substances which can be used medicinally. It is rich in minerals: silicon (5-10%), potassium and calcium [10]. Silicon is generally classified as an element which is important for plants for their structure, physiology and for protection [11]. Silicon is taken up by the roots in the form of silicic acid [Si(OH)₄], an uncharged monomeric molecule, when the solution pH is

below 9 [12]. Silicic acid is a soluble form of silicon and one of the basic forms, which is absorbed and used by plants. Polymerized silicates belong to the hardest materials in plant tissues. Silicon helps raise the plant health by the creating stronger and more resistant structures [23].

Alongside many other plant species, horsetails (*Equisetum* sp.) have been long recognized as remedies used for kidney troubles, rheumatic diseases, bleeding ulcer and rapid healing of bleeding wounds [10]. Si increases the resistance against mold. Recent researches noted that first response after fungi attack is higher in the presence of silicic acid [11].

The purpose of this study was to evaluate different types of Romanian *Equisetum* species extracts (*E. pratense*, *E. sylvaticum* and *E. telmateia*) for their antimicrobial activity and antioxidant capacity. In addition, the paper includes a qualitative TLC method and two spectrophotometric methods used to quantify Si content and total phenolic content in plant materials and extracts.

Experimental part

Plant materials

Sterile stems of *E. pratense*, *E. telmateia* and *E. sylvaticum* were used. *E. pratense* and *E. sylvaticum* were harvested from the North-Eastern region of Romania in June-July 2017. *E. telmateia* was collected in July 2018 also from the North-Eastern region of Romania. They were dried to a constant mass for 21 days in a single layer in a dark room at a controlled temperature of 23°C.

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Extract preparation

Two types of total extracts were obtained using different solvents: methanol 70% and ethyl-acetate [12, 22]. The methanolic extract was obtained from 10g of dry plant material using the water bath and the refrigerant for 60 minutes. The ethyl-acetate extract was obtained from 10g of vegetal product using a magnetic stirrer for three hours at room temperature. After filtration, the solvent was removed using a Buchi R-210 Rotavapor System at 40°C.

Determination of silicon content

The concentration of SiO_4 was determined using an adapted spectrophotometric method [1]. Determinations were made for both the stem and nodal branches. The sample contained 0.1g of dried plant material, 3mL NaOH 50% and 47mL distillate water claved at 121°C. 0.5mL from this sample was mixed with 17mL acetic acid 20%, 5mL ammonium molybdate solution (54 g/L, pH 7), 2.5mL tartaric acid 20% and 0.5mL reducing solution. Thirty minutes later, the absorbance was measured at 650nm [1]. The samples were prepared in triplicate and the mean value of absorbance was obtained. The concentration of silicon was expressed in SiO_2 equivalent ($\mu\text{g/mL}$). To avoid contamination of samples with external silicon in glass, the bottles, flasks and pipettes used in this study were made of polypropylene. All reagents used in this study were of analytical grade [13].

TLC determination

The qualitative chemical study was performed to evaluate total phenolic content in methanolic extracts [14]. 10 μL of methanolic solution from the three extracts was spotted on the silica gel 60 F₂₅₄ adsorbent. Also, standards like caffeic acid, chlorogenic acid, gallic acid and ferulic acid were spotted on the plat. For chromatography solvent was used the combination ethyl acetate-glacial acetic acid-formic acid- water (100:11:11:26). The detection was made UV at 365nm [15].

Total phenolic content

The amount of total soluble phenolics in extracts was determined spectrophotometrically according to the Folin-Ciocalteu method [2]. Using this method the OH groups can be evaluated from the studied sample in an alkaline medium (adjusted with sodium carbonate). The absorbance at the wavelength of 765nm, increases proportionally with the number of OH groups of the polyphenols [16]. The reaction mixture was prepared by mixing 0.04mL of methanolic and ethyl acetate solutions (concentration 5mg/mL) of extracts, 3.16mL of distilled water, 0.2mL of Folin- Ciocalteu reagent and 0.6mL of 20% sodium carbonate solution. After 2h of incubation the absorbance was measured at 765nm in spectrophotometer UV-VIS Jasco V-550. The total phenolic content was expressed as mg gallic acid per gram dry sample [2].

Antioxidant capacity (DPPH method)

Radical scavenging activity of plant extracts against stable 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) was determined by the reaction with an antioxidant compound which can donate hydrogen and reduce DPPH [17]. The change in color (from deep violet to light yellow) was measured at 517nm on UV-VIS spectrophotometer Jasco V-550 [25]. The stock solution of the plant extracts was prepared in DMSO to achieve concentrations of 20mg/mL, 10mg/mL, 5mg/mL and 2.5mg/mL. 0.05mL of these solutions were mixed with 2.95mL of DPPH methanolic

solution. The absorbance was measured using methanol as control after 300 s [3]. The experiment was carried out three times. Radical scavenging activity was calculated by the following formula:

$$\% \text{ scavenger activity} = \frac{(A_{\text{initial}} - A_{\text{final}})}{A_{\text{initial}}} \cdot 100$$

where: A_{initial} = absorption of blank sample ($t=0$ min), A_{final} = absorption of test extract solution ($t=300$ s). Gallic acid was used as positive control and the inhibitory concentration (IC_{50}) was estimated from the % inhibition versus concentration sigmoidal curve using a nonlinear regression analysis expressed as $\mu\text{g/mL}$ [3, 16, 17, 25].

Antimicrobial activity

The antimicrobial activities of methanol and ethyl-acetate extracts were tested against four selected microorganisms: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028). The experiment was carried out using a disc-diffusion method [18]. A small amount of each microbial culture was diluted in sterile 0.9% NaCl until the turbidity was equivalent to McFarland standard no. 0.5 (10^6 CFU/mL). The suspensions were further diluted 1:10 in Mueller Hinton agar for bacteria (Oxoid) and Muller Hinton agar for fungi (HiMedia) and then spread on sterile Petri dishes [19, 20]. Sterile stainless steel cylinders (5 mm internal diameter; 10mm height) were applied on the agar surface in Petri dishes. Then, 0.1mL of each extract was added into cylinders. Commercially available discs containing Ciprofloxacin (5 $\mu\text{g/disc}$), Fluconazole (25 $\mu\text{g/disc}$) and Nystatin (100 $\mu\text{g/disc}$) were used as positive controls. The plates were incubated at 37°C for 24 h. After incubation, the diameters of the inhibition zones were measured in mm, including disc size [19, 20].

Results and discussions

Determination of silicon content

The results for Si concentration in horsetail varied from 4.45% to 7.58% of the dry sample (table 1). The lowest amount of silicon was found in the stem of the plants in the range between 4.45% and 5.97% while the highest amount was found in the nodal branches in the range between 6.21 and 7.58%. The determinations revealed that *E. telmateia* contains the biggest concentration of SiO_2 , both in the stem (127.55mg/g dry plant) and in the nodal branches (162.10mg/g dry plant). *E. prantese* and *E. sylvaticum* contain similar but smaller concentration of SiO_2 ranging between 95.12mg/g and 101.5mg/g for the stem and 132.84mg/g and 146.96mg/g for the nodal branches.

TLC determination

Analysis of this chromatogram (fig. 1) revealed that a series of colored compounds in different fluorescent or dark blue nuances were identified from the methanol extracts.

Comparing the chromatogram results with the standards and the literature data [15], the following possible compounds were identified: Chlorogenic acid ($R_f \sim 0.4-0.5$), Gallic acid ($R_f \sim 0.6$) and Caffeic/Ferulic acid ($R_f \sim 0.8-0.9$) [24].

This TLC method demonstrated that the *Equisetum* species contain a very large amount of polyphenols which can be easily distinguished from other species of plants [21].

Samples	SiO ₂ mg/g dry plant	Si % (g/100g) dry plant
<i>Equisetum pratense</i> stem	101.5±5.21	4.745 %
<i>Equisetum pratense</i> nodal branches	132.84±3.37	6.210 %
<i>Equisetum sylvaticum</i> stem	95.12±4.16	4.446 %
<i>Equisetum sylvaticum</i> nodal branches	146.96±3.82	6.870 %
<i>Equisetum telmateia</i> stem	127.55±3.94	5.962 %
<i>Equisetum telmateia</i> nodal branches	162.10±3.25	7.578 %

Table 1
CONCENTRATION OF SILICON IN
Equisetum SPECIES

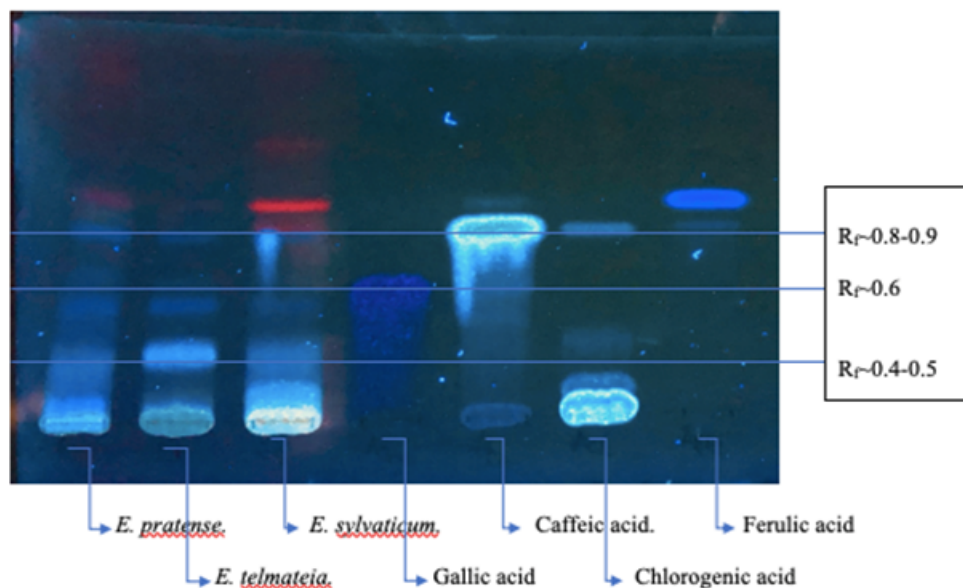


Fig.1. TLC determination

Total phenolic content

The content of total phenolic compounds of different horsetail extracts are shown in figure 2.

The obtained results showed that the concentration of total phenolic compounds in methanol 70% was significantly higher than in ethyl acetate for all three extracts. This fact is in correlation with polarity of the solvents used for extraction and solubility of phenolic compounds in them [22]. The highest concentration was determined for *E. sylvaticum*, methanolic extract, 196.50mg/g dry sample, while the lowest was obtained for *E. pratense* 74.13mg/g dry sample, also methanolic extract.

Antioxidant capacity (DPPH method)

The DPPH free radical scavenging potentials of methanolic extracts from three *Equisetum* species were investigated. It was found that all samples exhibited antioxidant activity; the *E. sylvaticum* extract possessed the best inhibitory activity (89.63%) at a concentration of 10mg/mL comparable to gallic acid used as control (fig. 3).

The IC₅₀ values of the analysed extracts and of the positive control reveal that *E. sylvaticum* extract presents the lowest value and therefore a good antioxidant activity (261.70µg/mL), while *E. telmateia* had an antioxidant capacity approximately two times lower (429.50µg/mL) as showed in figure 4.

These results qualify *E. sylvaticum*, *E. pratense* and *E. telmateia* as rich sources of natural antioxidants.

Antimicrobial activity

The in vitro results of antibacterial and antifungal activities of methanol 70% and ethyl acetate extracts of *E. pratense*, *E. sylvaticum* and *E. telmateia* are presented in table 2. These are expressed in mm as diameters of inhibition zones corresponding to the tested extracts.

The results indicate that all samples had activity against gram positive bacteria, *Staphylococcus aureus* (ATCC 25923). Of these extracts, the highest inhibition diameter is observed for *E. telmateia*, the methanolic extract (16mm), while for *E. pratense*, ethyl acetate extract was

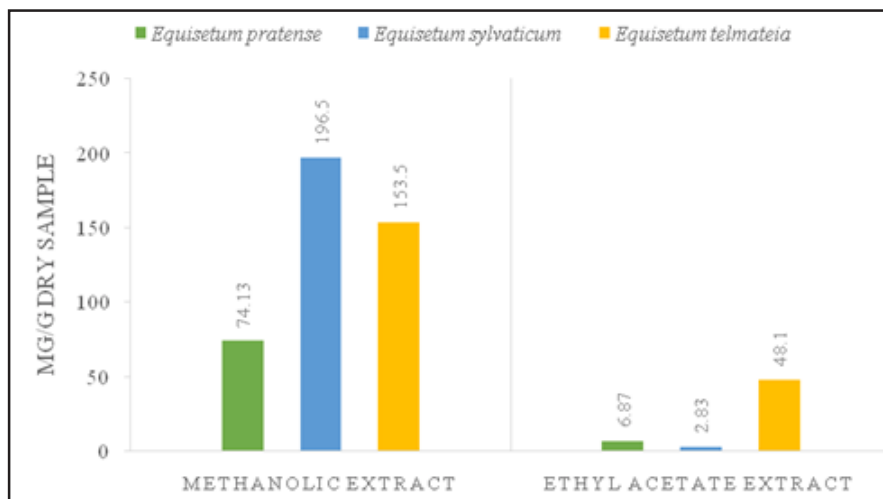


Fig. 2.Total phenolic content for
Equisetum species

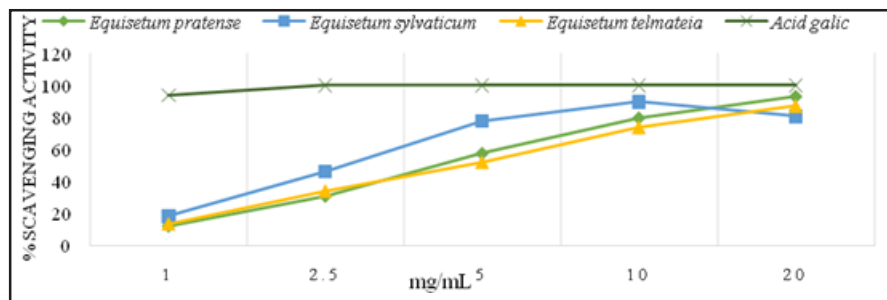


Fig. 3. Scavenging activity for *Equisetum* species and gallic acid

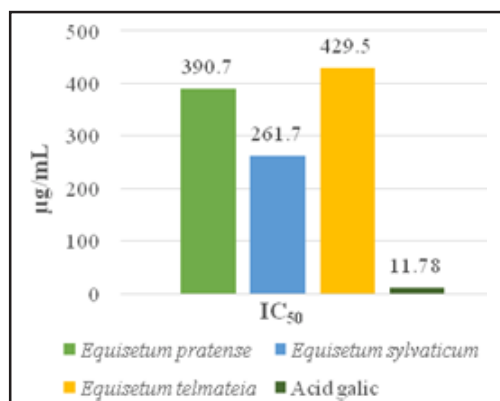


Fig. 4. IC₅₀ graphic for *Equisetum* species and gallic acid

Samples	Diameters of inhibition (mm)		
	<i>Staphylococcus aureus</i> (ATCC 25923)	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	<i>Candida albicans</i> (ATCC 90028)
<i>E. pratense</i> 1	14	0	12
<i>E. pratense</i> 2	10	9	11
<i>E. sylvaticum</i> 1	10	0	11
<i>E. sylvaticum</i> 2	13	9	12
<i>E. telmateia</i> 1	16	0	16
<i>E. telmateia</i> 2	14	9	14
Ciprofloxacin	28	28	-
Nystatin	-	-	20
Fluconazole	-	-	30

1- Methanolic extract; 2- Ethyl acetate extract

Table 2
DIAMETER OF INHIBITION ZONES (mm) OF THE SAMPLES AND CONTROLS

obtained the smallest diameter (10mm). Against *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), gram negative bacteria, the tested extracts demonstrated weak to no antimicrobial activity. All samples showed good activity against *Candida albicans* (ATCC 90028). The extracts obtained from *E. telmateia*, especially the methanolic extract, had the highest inhibition diameter developing the most pronounced antifungal activity (16mm) [11, 26].

Conclusions

The results from this study portray horsetail extracts as an easily accessible source of high content of natural Si. Furthermore, they possess a very good antioxidant activity due to the high total phenolic content. The highest antimicrobial efficacy was for *E. telmateia* predominantly in *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028) strains. This activity may be correlated with the high content of Si that these ferns possess. This research can provide useful information for screening this plant as a potential source of bioactive components with antioxidant properties that may be

included in dietary supplements and helpful in preventing different disease.

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