

TLC, makroskopska, mikroskopska analiza vrsta roda *Equisetum*

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SAŽETAK: Poljska preslica je u narodu poznata i kao rastavić, konjski rep, kositrena trava. Rasprostranjena je skoro po cijeloj sjevernoj Zemljinoj polulopti. Ima dvije vrste izdanaka, fertilni i sterilni.

Aktivnosti pri izradi ovog rada zahtijevale su terenski i laboratorijski rad. U terenskom dijelu rada prikupljen je biljni materijal sa šest različitih lokaliteta na području općine Živinice tokom mjeseca jula 2016. godine. Svjež biljni materijal je konzerviran u FOA konzervansu za mikroskopsku analizu, dok je drugi dio biljnog materijala za makroskopsku i analizu tankoslojnom hromatografijom osušen na provjetrenom mjestu. Makroskopska identifikacija izvršena je golim okom i uz pomoć povećala, te su uz upotrebu priručnika za određivanje biljaka determinisane vrste s odabranih lokaliteta. Mikroskopska identifikacija izvršena je svjetlosnim mikroskopom, a TLC analiza prema standardnoj proceduri za detekciju flavonoida.

Identifikovane su vrste: *Equisetum arvense* L. (prisutne na četiri lokaliteta) i *Equisetum telmateia* Ehrh. (prisutne na dva lokaliteta). Okarakterisana je i detaljno opisana njihova makroskopska i mikroskopska građa. TLC-om je detektovano prisustvo flavonoida u herbi *Equisetum arvense* L. (hlorogenske kiseline i drugih) u znatno većoj količini u odnosu na herbu *Equisetum telmateia* Ehrh. Ljekoviti potencijal poljske preslice je izuzetno veliki. U upotrebi je dugi niz godina, ali i dalje plijeni pažnju istraživača te se svakodnevno iznalaze njene nove terapijske mogućnosti.

Ključne riječi: TLC analiza, *Equisetum*, mikroskopska, makroskopska identifikacija

TLC, macroscopic and microscopic analysis of species of the genus *Equisetum*

SUMMARY: Field horsetail is also known as scouring rush, horse willow and shave grass. Field horsetails are to be found throughout the northern hemisphere. They produce two types of shoots: fertile and sterile.

The research required field and laboratory investigations. With regard to field work of the research, the plant material was collected from six different sites in the Municipality of Živinice during July 2016. The fresh plant material was preserved in FAE preservative for the microscopic analysis, and another part of the plant material was dried in a well-ventilated area for the macroscopic and Thin Layer Chromatography analyses.

The macroscopic identification was conducted using the naked eye as well as a magnifying glass, and the species from the selected sites were identified using a handbook for plant determination. The microscopic identification was conducted using a light microscope, while TLC analysis was done according to the standard procedure for the detection of flavonoids.

The following species have been identified: *Equisetum arvense* L. (present at four sites) and *Equisetum telmateia* Ehrh. (present at two sites). Their microscopic and macroscopic structure has been characterized and described in detail. TLC analysis has revealed the presence of flavonoids in significantly larger quantities in *Equisetum arvense* L. herb (chlorogenic acids and others) in comparison to *Equisetum telmateia* Ehrh herb. Medical potential of field horsetail is significantly high. Even though it has been used for a long time, field horsetail still attracts researchers' attention and its new therapeutic possibilities are constantly discovered

Key words: TLC analysis, *Equisetum*, microscopic, macroscopic identification

INTRODUCTION

***EQUISETUM ARVENSE* L. – field horsetail**

Equisetum arvense L. – field horsetail belongs to the order *Equisetales* which has one extant family *Equisetaceae* with one surviving genus - *Equisetum*. Field horsetail is commonly known as scouring rush, horse willow and shave grass. It grows throughout the northern hemisphere and produces two types of shoots: fertile and sterile. Sterile aerial parts of the plant are collected for medicinal purposes (*Equiseti*

herba). Properly dried horsetail keeps its green color. It does not have any special odor and its taste is mild, somewhat bitter and salty (Bosnić, Pekmić, Huseinović, Osmanović, 2011; Ferhatović, Mišić, Medžedović, 2003).

The chemical composition of field horsetails varies due to their habitats and areas in which they grow. They are specific due to a high percentage of silicic crystals. The total content of silicic acid increases with the age of a plant, however, in the process of collecting, young plants should have the priority. Although they have a lower content of the total silicic

acid concentration, they have a higher content of soluble silicic acid. That soluble creamy (silicic) acid creates a suitable curative effect.

Field horsetail contains 10 – 22 percent of minerals (ash): silicic acid (70 – 80 %), potassium (around 1.5%), calcium (1.3 %) and aluminum. The main active substances in field horsetail are: the saponin equisetin, aconitic acid, salts of creamy acid, alkaloids (equisetin - palustrine, nicotine, 3-methoxy-pyridine), flavonoid compounds (heteroside, quercetin, equisetin) and antithiamine active substances (plant thiamine, caffeic acid). It also contains a wide range of organic acids, aconitic, oxalic, malic and tannic acid; polyenic acids, tannins, resins, beta-sitosterol, cholesterol (around 3%), enzymes, vitamin C and carotenoids (Mägdefrau, Ehrendorfer, 1997; Solemani, Azarbaizani, Nejati, 2007).

Therapeutic properties of horsetail were known in ancient times. According to Dioscorides, the plant named horsetail as a plant which stanches bleeding, soothes coughs and increases the secretion of urine. Horsetail prevents stone formation. It is used to treat urinary tract infections and to stop bleeding. More recently, it has been confirmed that horsetail is a diuretic and an astringent. Horsetail has been proven to have diuretic effect in animal studies. Water diuresis takes place, and because of its mechanism of action horsetail is considered an aquaretic (Husejinovic, Bektić, Lolić, 2019).

Equiseti herba – field horsetail herb is official in the German Pharmacopoeia DAB 10, the Austrian Pharmacopoeia OAB 90, the European Pharmacopoeia Ph.Eur.Supplement 4.2 (2002). The pharmacopoeias that provide monographs of field horsetail require the identification investigation using the Thin Layer Chromatography method.

Marsh horsetail, *Equisetum palustre*, can sometimes be confused with field horsetail due to its high similarity to field horsetail. However, marsh horsetail is poisonous to animals because of the high content of alkaloid palustrine (4).

EQUISETUM TELMATEIA Ehrh. (E.max.) – great horsetail

Great horsetail is the largest of all the species of the genus *Equisetum*. The fertile and sterile shoots are different and they grow separately. The fertile shoots are produced in March and they die after spore release. The sterile shoots start growing in April and May and can be up to two meters tall. Great horsetail is used to remedy urinary tract infections, lung disorders and to stop bleeding (Kuštak, 2005; Mišić, Lakušić, 1990).

The aim of the paper is to identify the species of the genus *Equisetum* which grow in the area of the Municipality of Živinice, and to improve the knowledge about their basic anatomical-morphological characteristics as well as the therapeutic properties of these plants.

MATERIAL AND METHODS

The plant material was collected in July 2016 from six sites in the Municipality of Živinice where the plants are naturally found. The following sites were included in the research: Spreča, Višća, Križaljka, Barice,

Bašigovci and Dubrave. The collected fresh horsetail stems were preserved in FAE (formaldehyde, acetic acid and ethyl alcohol) preservative. The other part of the plant material was dried in a well-ventilated area in a thin layer.

Laboratory work included the macroscopic identification of the plant material using the naked eye, a magnifying glass and a handbook for plant identification and the microscopic analysis of the plant material using a microscope. Finally, TLC (Thin Layer Chromatography) was done to detect the presence of flavonoids in it.

Thin Layer Chromatography is a basic separation technique described in pharmacopoeias and is primarily used for qualitative and quantitative analysis. This technique is used to separate and determine naturally occurring compounds, and to obtain characteristic spots of plant drugs that contain different phytoconstituents (Petrović, Maksimović, Kundaković, 2009). Investigation of the presence of flavonoid heteroside in aerial parts of *Equisetum arvense* L. and *Equisetum telmateia* Ehrh. was conducted using Thin Layer Chromatography with the following procedure.

The test solutions are dried and powdered aerial stem parts of *Equisetum arvense* L. and *Equisetum telmateia* Ehrh. (1.00 g) which are covered by 10 ml of methanol and heated in a water-bath at 65°C for 10 min, with frequent agitation. After heating, they are left at room temperature to cool down and filtrate.

The reference solutions are chlorogenic acid and rutin. *Stationary phase* includes aluminum plates with a thin layer of silica gel and fluorescent indicator (Silica gel 60 F₂₅₄).

Mobile phase includes ethyl acetate – anhydrous formic acid – glacial acetic acid – water (100:11:11:26). Then, 10 µl bands of solution are applied.

Detection: the plates are dried at 100-105°C and while they are warm they are treated with NP-reagent (a solution of diphenylboric acid aminoethyl ester) (10g/l), and then they are treated with the same amount of a solution of macrogol 400 (50g/l). The plates are air-dried (for about 30 min). The obtained chromatogram with fluorescent zones is investigated in UV light at 365 nm (Mujić, 2012).

RESEARCH RESULTS AND DISCUSSION

At four out of six investigated sites in the area of the Municipality of Živinice (Spreča, Križaljka, Barice i Dubrave) *Equisetum arvense* L. – field horsetail was present (Figure 1). At the two remaining sites (Višća and Bašigovci), *Equisetum telmateia* Ehrh.- great horsetail was present (Figure 2)

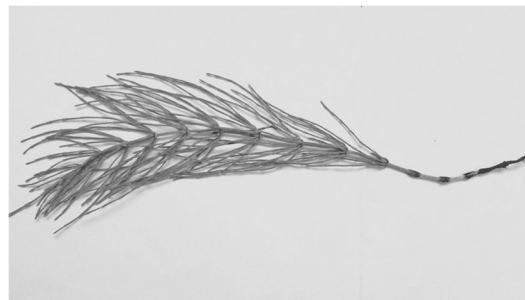


Figure 1. *Equisetum arvense* L., site – Križaljka (Original photograph by Arnaut E.)



Figure 2. *Equisetum telmateia* Ehrh., site – Višća
(Original photograph by Arnaut E.)

Similar research was conducted in Tuzla and its surroundings in 2012, and it included five sites. After the identification of the collected horsetail samples, it has been determined that *Equisetum arvense* L. grows in the area of Orahovica and Šići, *Equisetum telmateia* Ehrh. in Lukavac and Slavinovići, and *Equisetum palustre* L. can be found in Babice (Mujić, 2012).

Based on the macroscopic analysis of plants with the naked eye, a magnifying glass and handbook for plant identification, it has been determined that *Equisetum arvense* L. has grooved stem fragments and linear leaves, and its color is from light green to greenish-gray. The main stem of horsetail is about 0.8 to 4.5 mm in diameter, hollow; vertical grooves are present on the internodes ranging in number from 4 to 14. Branches are erect, each about 1 mm thick with 2 to 4 longitudinal grooves. The leaves are small, linear, and they form a toothed sheath around the stem. The number of teeth corresponds to the number of grooves on the stem. Each tooth is triangular and brown. The lowest internode of each branch is longer than the sheath of the stem it belongs to (Figure 3).

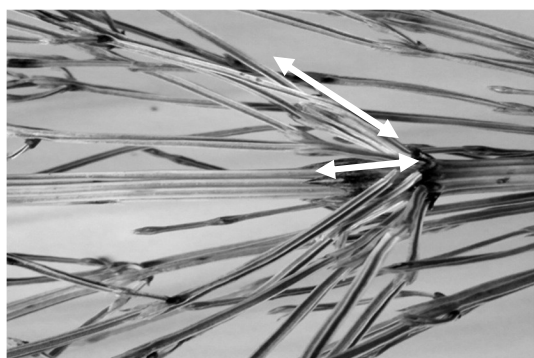


Figure 3. *Equisetum arvense* L. site: Dubrave
(Original photograph by Arnaut E.)

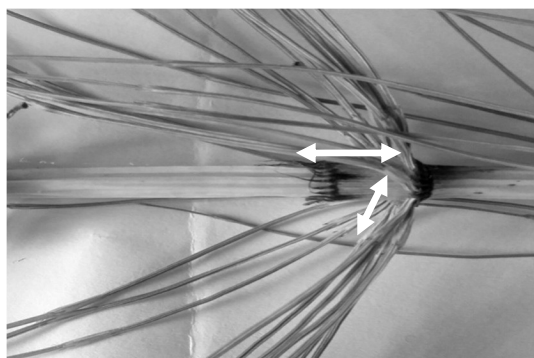


Figure 4. *Equisetum telmateia* Ehrh., site: Višća
(Original photograph by Arnaut E.)

In *Equisetum telmateia* Ehrh., the lowest internode of each branch is shorter than subtending stem sheath. The stem of great horsetail is off-white with numerous soft ascending branches; there are more than 14 sheath teeth (Figure 4).

With regard to the microscopic structure of horsetails, it is known that they have articulate stems with sets of canals. In the parenchyma cortex, there are vallecular canals, while in the central cylinder there is a big central cavity which takes from one to two thirds of the stem diameter (Figure 5).

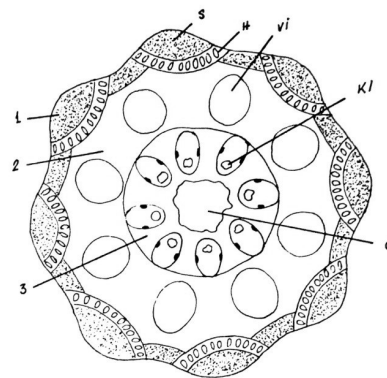


Figure 5. Cross-section through the stem of field horsetail, *Equisetum arvense* (schematic)

1 - epidermis; 2 - cortex; 3 - central cylinder; s - sclerenchyma (mechanical tissue); h - chlorenchyma (photosynthetic tissue); vi - vallecular canals; ki - carinal canals arranged in collateral vascular bundles; ci - central cavity

In vascular bundles, due to the disintegration of xylem a carinal cavity is formed. The stem is both articulate and ridged. It consists of ridges (*carinae*) and vallecules (*valleculae*). Below epidermis, whose walls have a deposit of siliceous material, there is cortex which is composed of sclerenchyma (mechanical tissue) with high amount in ridges, chlorenchyma (photosynthetic tissue) which are arranged arch-like below sclerenchyma and parenchyma of primary cortex where there are vallecular canals. The central cylinder is separated by an endodermis.

C₁ C₂ C₃ C₄ C₅ C₆

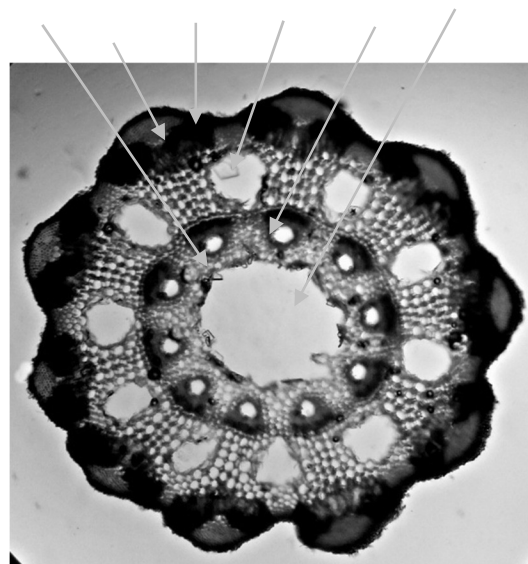


Figure 6. - *E. arvense* L., site – Barice (Original photography by Arnaut E.)

C₁ – central cylinder;
C₂ - sclerenchyma
C₃ - chlorenchyma
C₄ – valecular canals
C₅ – carinal canal
C₆- central cavity

It has collateral vascular bundles (arranged under ridges - carina) with the disintegrated xylem (carinal cavity), and it is surrounded by phloem (Pellag,Pasca, Tunde, Suci, Nemeth, Vikas, 2015).

The cross-section through *Equisetum arvense* L. from Barice and the stem of *Equisetum telmateia* Ehrh. from Višća analyzed under a microscope are given in Figures 6 and 7 with noticeable characteristic structure of horsetail stem and the difference between these two species, especially in terms of sclerenchyma and chlorenchyma.

The prepared samples of horsetail were investigated to detect the presence of flavonoids using Thin Layer Chromatography (TLC). Flavonoids belong to a group of polyphenolic compounds and are ubiquitously found in plants. They are secondary metabolites, which means that they belong to organic compounds that do not directly affect the growth and development of plants but have a significant role in their medicinal activity (Bosnić, Pekmić, Huseinović, Osmanović, 2011; Badole, Kotwal, 2014). After conducting Thin Layer Chromatography, whose process was previously described, the obtained results were photographed (Figure 8).

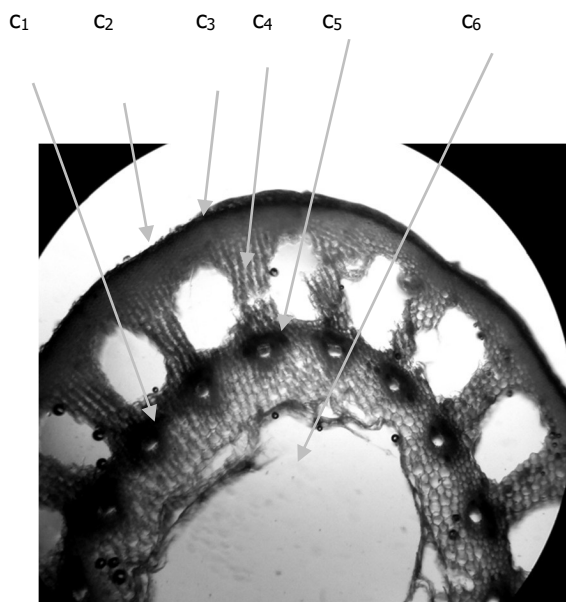


Figure 7. *E. telmateia* Ehrh., site: Višća (Original photography by Arnaut E.)

e₁ – central cylinder
e₂ - sclerenchyma
e₃ - chlorenchyma
e₄ – valecular canals
e₅ – carinal canals
e₆ - central cavity

Flavonoids in the chromatogram are emitted se as the light blue and yellow-orange fluorescent zones which in the case of great horsetail *Equisetum telmateia* Ehrh. are not present or are mildly visible while in *Equisetum arvense* L. they are present in great intensity. Based on the result obtained from the photographs, it can be concluded that *Equisetum arvense* L. is much richer in flavonoids than *Equisetum telmateia* Ehrh.

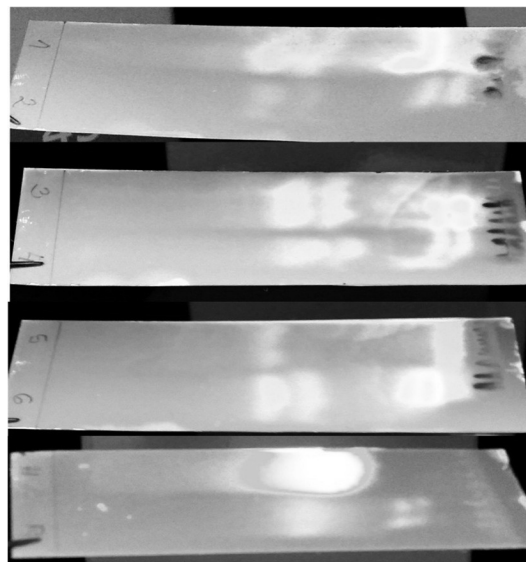


Figure 8. Chromatogram in ultraviolet (UV) light at 365 nm (Original photography by Arnaut E.)

1 – *Equisetum arvense* L., 2 – *Equisetum telmateia* Ehrh., 3 – *Equisetum arvense* L.,
4 – *Equisetum arvense* L., 5 – *Equisetum telmateia* Ehrh., 6 – *Equisetum arvense* L.,
C – chlorogenic acid, R – rutin

The reference solution consists of chlorogenic acid - C (the light blue fluorescent zone) and rutin - R (the yellow-brown fluorescent zone).

The presence of flavonoids in aerial parts of *Equisetum arvense* L. significantly contributes to its therapeutic potential. Unlike field horsetail, the *Equisetum telmateia* Ehrh. species did not show any significant content of flavonoids.

The chromatograms of *E. arvense* and *E. palustre* for the reference substances rutin, hyperoside and caffeic acid are shown in Figure 9. The difference in the flavonoid content for these two plant species is evident, although it is registered that *E. palustre* is used as a counterfeit for *E. arvense*.

The high antioxidant power of *Equisetum* was proved in a research's paper in which are examined field's *Equisetum* on the area of city Banja Luka 2012 year. The content of total phenols by the Folin Ciocalteu modified method and the antioxidant activity by the DPPH, FRAP and ABTS method were determined through research. The results of the study showed that ethanolic extract of *E. arvense* L. showed very significant antioxidantability in accordance with the total phenolic content (Zelenković, 2012).

The ethanolic extract of field's *Equisetum* tested in this research shows strong inhibitory's effect on *E.coli*, *S.aureus* and *B.cereus*. Also is proved and strong

inhibitory power effect against different species of bacterias which are cause of infections of urinary system that confirming the fact that field *Equisetum* has very significant antimicrobial properties. There are differences in the content bioactive substances of plant *E. arvense* collected on different geographical fields. The results of research field's *Equisetum* collected in an urban area Banja Luka, which is exposed to different types of pollutions are compared with investigated field *Equisetum* collected on area of the mountain Zlatibor (Serbia).

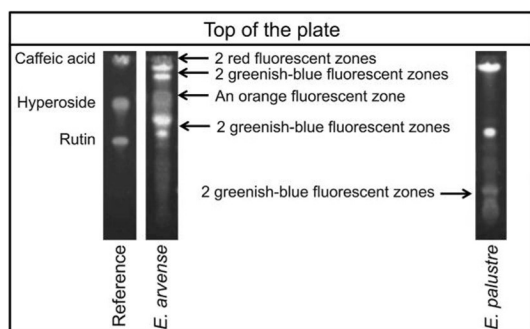


Figure 9. Chromatograms of *E. arvense* and *E. palustre* for rutin, hyperoside and caffeic acid

A field *Equisetum* collected on area of the mountain Zlatibor resulted with very high phenol's component that is difference of the field's *Equisetum* which is collected on the field of an urban areas whose results showed very poor antioxidant's content and phenolic activity relative to *Equisetum* which growing in unpolluted areas (Zelenković, 2012).

According to the research fields *Equisetum* conducted in Iran it was concluded that *E. arvense* L. has a significant role in the fight against diabetes. Methanole's extract, showed significant antihyperglycemic activity in streptozotocin aroused hyperglycemic mice with very significant changing in body weight. Histopathological examination together with biochemical examination suggests the possibility of regeneration of Langerhans's channel using the treatments of plant extract. (Soleimani, Azarbaizani, Nejadi, 2007).

In addition to that, in Australia used to treat rheumatism and diseases of the locomotor system. (Badole, Kotwal, 2014).

Research on field horsetail (*E. arvense* L.) and great horsetail (*E. telmateia* Ehrh.) in Romania (Palag, Pasca, Tunde, Suci, Nemeth, Vikas, 2015) in 2015 also indicated that the content of flavonoids was higher in field horsetail than in the case of great horsetail (Domac, 1994). HPTLC (High Performance Liquid Chromatography) is a more accurate and reproducible method in comparison to TLC. The fingerprints of *E. arvense* in tracks 4 and 5 and the fingerprints of the reference substances in tracks 1,2,3,6 and 7 can be observed (Figure 10). The separation of the zones is far better as well as the picture quality.

Table 1 presents the basic differences between the *E. arvense* L. and *E. telmateia* Ehrh species which have been determined throughout this research.

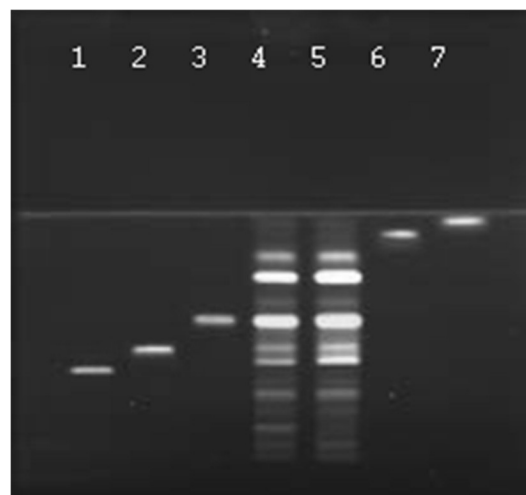


Figure 10. 1:Rutin, 2: Chlorogenic acid, 3: Isoquercitrin, 4:*Equisetum arvense*, 5:*Equisetum arvense*, 6: Caffeic acid, 7: Kaempferol; (11)

Table 1. Basic differences between the analysed *E. arvense* L. and *E. telmateia* Ehrh. Species

$Y = ax + b$	<i>E. arvense</i> L.	<i>E. telmateia</i> Ehrh.
Habitat	wet meadows, on embankments, road sides	along streams, swamps, woodland edges
Stem height	10 - 80 cm	15 - 200 cm
Stem color	light green/greenish gray	off-white
Stem diameter	0.8 – 4.5 mm	to 1.5 cm
Number of teeth	6 – 12	20 - 30
Lowest internode	longer than the stem sheath	shorter than the stem sheath
Amount of silicified collenchymas	more pronounced	less pronounced
Central cavity	narrow	wide
Number of vallecular canals	10 -12	18
Side branches in cross-section	10	not present
TLC	abundant in flavonoids	deficient in flavonoids
Use in pharmacy	yes	no
Presence in the area of the Municipality of Živinice	four sites	two sites

CONCLUSION

Research on the genus *Equisetum* in the area of the Municipality of Živinice was conducted at seven sites in July this year. The *Equisetum arvense* L. species was

identified at the following sites: Spreča, Križaljka, Barice and Dubrave. The *Equisetum telmateia* Ehrh. species was identified in Višća and Bašigovci.

The macroscopic identification showed that the distance between internodes in *Equisetum arvense* L. is longer than the stem it belongs to. Stem sheath teeth are dark with inconspicuous light margins, often cohering in pairs and ranging in number from 10 to 12, equaling the number of stem grooves. Cross-section through the stem shows the central canal with vallecular and carinal canals.

Choice method for the identification of plant drug is Thin Layer Chromatography. Using TLC method, the presence of flavonoids in field horsetail *E. arvense* L. was confirmed, while in great horsetail *E. telmateia* Ehrh. flavonoid content was deficient, which was also confirmed by other investigations conducted on these plants.

The European Pharmacopoeia only includes the *Equisetum arvense* L. species as a source of medicinal drug *Equiseti herba*, and it is of great importance to conduct a detailed investigation of plant material which also includes the microscopic research in order to confirm that it is field horsetail.

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