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Antioxidant, Antimicrobial and Genotoxicity Screening of Hydro-alcoholic Extracts of Five Serbian *Equisetum* Species

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Abstract The hydro-alcoholic extracts of five *Equisetum* species, *E. arvense* L., *E. sylvaticum* L., *E. fluviatile* L., *E. palustre* L. and *E. telmateia* Ehrh., growing-wild in Serbia were evaluated for their genotoxicity, antimicrobial activity, antioxidant capacity and the results related to the total phenol content and HPLC flavonoid profiles. The total phenol content was 92–349 μmol expressed as equivalents of chlorogenic acid per g of dried plant material. Main identified compounds were kaempferol-, quercetin- glycosides and caffeic acid derivatives. *E. telmateia* extract showed the greatest antioxidant capacity. Almost all tested microorganisms demonstrated some degree of sensitivity to the examined extracts. All tested extracts at 62.5 μg/ml showed higher incidence of micronucleus formation than in the control sample. The obtained data allowed mutual comparison of examined species and their assessment as possible sources of antioxidants, antimicrobials and/or genotoxic substances.

Keywords Antimicrobial activity · Antioxidant capacity · *Equisetum* sp. · Flavonoids · Genotoxicity · Hydro-alcoholic extracts

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Introduction

According to Flora Srbije [1], there are five species of the subgenus *Equisetum* indigenous to Serbia, *E. arvense* L., *E. sylvaticum* L., *E. fluviatile* L., *E. palustre* L. and *E. telmateia* Ehrh. All other horsetails, except *E. palustre* (marsh horsetail) which is a well-known livestock poison, containing a poisonous ingredient—alkaloid palustrine, are locally used in human diet, for diuretic preparations and wool coloring. Field horsetail (*E. arvense* L.) sporophyte (*tsukushi*) is consumed as food in sweetened vinegar, cooked food, and chopped fish, while nutritive caulis (*sugina*) is well known as *Sugina* tea in Japan and is drunk as a health drink [2]. Some plants used as foodstuff also have a role in the maintenance of human health and protection from a variety of disease. Alongside with many other plant species, horsetails (*Equisetum* sp.) have been long recognized as folk remedies used for kidney troubles, arthritis, bleeding ulcers, and tuberculosis and also to stop the bleeding of wounds and promote rapid healing [3]. Recent findings have even demonstrated that the hydro-alcoholic extract of the well-known *E. arvense* is active towards the central nervous system [4]. Among 15 living species of this genus and a number of hybrids [5] only *E. arvense* (as "Equiseti herba") is listed in a number of European pharmacopoeias.

The purpose of this study was to evaluate the hydro-alcoholic extracts of five Serbian *Equisetum* species, *E. arvense* L., *E. sylvaticum* L., *E. fluviatile* L., *E. palustre* L. and *E. telmateia* Ehrh., for their genotoxicity, antimicrobial activity and antioxidant capacity and to relate the obtained results with the total phenol content and HPLC flavonoid profiles, as well as to assess and compare these species as possible sources of antioxidants, antimicrobials and/or genotoxic substances. To the best of our knowledge, *E. sylvaticum*, *E. fluviatile* and *E. palustre* have not been tested in the micronucleus test, antimicrobial or antioxidant capacity assays. Previously, only Equiseti herba was evaluated in the micronucleus test [6]. Antioxidant activity of *E. arvense* and *E. telmateia* extracts [2, 7, 8] and the antimicrobial activity of the essential oil of *E. arvense* [9] have been reported. HPLC profiles of methanolic extracts of *E. arvense*, *E. sylvaticum*, *E. fluviatile*, *E. palustre* and *E. telmateia* [10, 11] were previously investigated, likewise the profile of the ethyl acetate fraction of *E. telmateia* infusion has been published recently [8].

Materials and Methods

Plant Materials

The sterile stems of all *Equisetum* species were collected in S.E. Serbia, in August 2005, (*E. arvense, E. palustre* and *E. sylvaticum* from the marshes of lake Vlasina, *E. fluviatile* from the banks of river Vlasina and *E. telmateia* from the banks of river Dušnička reka) and authenticated by Dr. V. Ranđelović (Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Serbia). Voucher specimens have been deposited in the Herbarium of the Faculty of Biology, University of Belgrade, Serbia (BEOU, *E. arvense* No. 16072, *E. fluviatile* No. 16073, *E. palustre* No. 16074, *E. sylvaticum* No. 16075 and *E. telmateia* No. 16076). The plant material was air-dried, at room temperature, before extract preparation.

Preparation of Extracts

Plant extracts preparation for all assays and the measurements of the total phenols were done according to the published procedure [12]. Briefly, dried and milled plant material (3.0 g) was weighed and transferred to a heavy duty Waring blender equipped with a stainless steel water-jacketed jar. Eighty milliliters of 80% (*v*/*v*) ethanol was added and the mixture was blended for 8.0 min then vacuum filtered through No. 541 Whatman filter paper. The solids were re-extracted with 60 ml of 80% ethanol and the filtrates combined and made to a final volume of 250 ml. Three replicate extractions of each sample were made. Ten milliliters of each extract was filtered through a 0.45 μm membrane filter and kept in a capped vial at 0°C for the measurement of phenolics. For other measurements, 200 milliliters of each extract were evaporated to dryness *in vacuo* at 45°C. Percent residue content (mass of residue per mass of dried plant material, w/w) is given in Table 2.

Determination of Total Phenols

Total phenolics were measured using a modified version of Glories' method [12]. In a test tube, 0.25 ml of 0.1% HCl in 95% ethanol, 4.55 ml of 2% HCl and 0.25 ml of standard solutions of chlorogenic acid (Sigma Chemical Co., St. Louis, USA) or filtered extract made up in 95% ethanol was added, mixed by vortex and allowed to stand for approximately 15 min before reading the absorbance at 280 nm. The total phenol content was calculated and expressed as μmol of chlorogenic acid per gram of dry matter or dry extracts (residue) using a standard curve at five concentrations covering the range, 50–1,600 μmol.

HPLC Analysis

The HPLC analyses were performed on an Agilent 1100 Series HPLC System with Micro Vacuum Degasser, Binary Pump, thermostatted Column Compartment and Variable Wavelength Detector. Operating conditions were the same as in Veit et al. [11]. Column: Agilent Eclipse XDB-C18 4.6 mm ID×150 mm (5 μm) 80 Å. Elution profile: A=0.15% phosphoric acid in H_2O–MeOH 77:23 (*v*/*v*, pH=2); B= MeOH. Isocratic: 0–3.6 min 100% A; gradient: 3.6 min 100% A-linear-24.0 min; 80.5% A-isocratic-30 min linear-60 min; 51.8% A-linear-67.2 min; 100% B; flow rate: 1.0 ml/min. The injected volume was 20 μl. Spectrophotometric detection in the UV region at 350 nm was used; therefore all used solvents had to have sufficient light permeability at this wavelength. Due to the poor reproducibility of retention times in HPLC chromatograms, we calculated the relative retention indices (as the ratio between retention time of the component in question and naringenin as the internal standard). The peak identity was checked by comparison of their relative retention indices with the previously published ones [11] and by co-injection with naringenin, quercetin, kaempferol, apigenin and rutin (Sigma Chemical Co. St. Louis, USA). The percentage composition of the extracts (Table 1) was computed from the HPLC peak areas.

Antioxidant Capacity

The total antioxidant capacity of the extracts was evaluated by the method of Prieto et al. [13] using a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer. Briefly, an aliquot

Table 1 Flavonoids and caffeic acid derivatives of the hydro-alcoholic (20:80, v/v) extracts of five *Equisetum* species (relative peak area %)

Compound ↓	Plant →				
	E. arvense L.	*E. sylvaticum* L.	*E. fluviatile* L.	*E. palustre* L.	*E. telmateia* Ehrh.
Apigenin	–	–	2.9	–	–
Apigenin 4'-*O*-glucoside	–	–	3.7	–	–
Quercetin	21.1	–	–	–	–
Quercetin 3-*O*-glucoside	49.6	44.7	–	–	–
Quercetin 3-*O*-(6''-*O*-malonylglucoside)	8.8	–	–	–	–
Kaempferol	–	–	–	4.7	1.6
Kaempferol 3-*O*-glucoside	–	7.9	4.6	11.9	6.9
Kaempferol 3-*O*-(6''-*O*-malonylglucoside)-glucoside	–	–	7.9	–	
Kaempferol 3-*O*-(6''-*O*-acetylglucoside)	–	–	–	–	10.4
Kaempferol 3'-*O*-rutinoside	–	1.9	–	3.3	–
Kaempferol 3,7-*O*-diglucoside	–	7.6	47.6	–	23.6
Kaempferol 3-*O*-(6''-*O*-acetylglucoside)-7-*O*-glucoside	–	–	–	–	12.7
Kaempferol 3-*O*-glucoside-7-*O*-rhamnoside	–	12.5	–	–	2.5
Kaempferol 3-*O*-(6″-*O*-acetylglucoside)-7-*O*-rhamnoside	–	–	–	–	10.1
Kaempferol 3-*O*-rutinoside-7-*O*-sophoroside	–	–	–	24.7	–
Kaempferol 3-*O*-rutinoside-7-*O*-glucoside	–	5.3	–	12.3	4.9
5-*O*-Caffeoyl shikimic acid	4.4	4.7	19.0	–	18.4
Monocaffeoyl *meso*-tartaric acid	3.0	4.5	10.4	2.5	2.2
Dicaffeoyl *meso*-tartaric acid	1.6	–	–	–	–

of 0.1 ml of sample solution (1 mg of extract per ml of methanol) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonim molybdate). The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. A blank solution contained 1 ml of reagent solution and 0.1 ml of methanol, and it was incubated under the same conditions as the samples. Stock solutions of α-tocopherol acetate were prepared in methanol just prior to use. The total antioxidant capacity was expressed as equivalents of α-tocopherol acetate (μmol per gram of extract) using standard curve at five concentrations covering the range, 50–1,600 μmol. Exact concentrations were determined spectrophotometrically on the basis of the absorption coefficients from literature.

Antimicrobial Activity

The *in vitro* antimicrobial activity of the extracts against a panel of laboratory control strains belonging to the American Type Culture Collection Maryland, USA: Gram-positive: *Staphylococcus aureus* (ATCC 6538), Gram-negative: *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13076); fungal organisms *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231) and the Gram-negative bacteria *Escherichia coli* 95 (origin—Institute of Immunology and Virology "Torlak") were determined using a disk diffusion method according to the previously described procedure [9].

The following nutritive media have been used: Antibiotic Medium 1 (Difco Laboratories, Detroit, Michigan, USA) for growing Gram-positive and Gram-negative bacteria and Tripton soy agar (TSA-Torlak, Belgrade) for *Candida albicans* and *Aspergillus niger*. Nutritive media have been prepared according to the instructions of the manufacturer. All agar plates were prepared in 90 mm Petri dishes with 22 ml of agar giving the final depth of 4 mm. 0.1 ml of a suspension of the tested microorganisms (10^8 cells per ml) was spread on the solid media plates. Sterile filter paper disks ("Antibiotica Test Blattchen", Schleicher and Schuell, Dassel, Germany, 6 mm in diameter) were impregnated with 50 μl of the extracts (all extracts were filter-sterilized using a 0.45 μm membrane filter) and placed on inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the fungi. Standard disks of Ampicillin and Nystatine (origin—Institute of Immunology and Virology "Torlak", 30 μg of the active component, diameter 6 mm) were individually used as positive controls, while the disks imbued with 50 μl of pure methanol as a negative control. The diameters of the inhibition zones were measured in

Table 2 Extract yield, total phenol content, antioxidant capacity, antimicrobial activity and effect on the incidence of micronuclei formation of the hydro-alcoholic (20:80, *v*/*v*) extracts of five Serbian *Equisetum* species

Assay ↓	Plant →				
	E. arvense L.	*E. sylvaticum* L.	*E. fluviatile* L.	*E. palustre* L.	*E. telmateia* Ehrh.
Extract yield (%, *w*/*w*)[a]	8.7	22.8	10.4	14.7	16.0
Total phenol content (μmol chlorogenic acid per g of dry plant material or dry extract, values in parentheses)[a]	92(1070)	349(1531)	127(1221)	156(1061)	216(1350)
Antioxidant capacity, equivalents of α-tocopherol acetate (μmol per g of extract)[b]	104867± 101	44733±49	1793±29	1652±94	133463± 111
Zones of inhibition *P. aeruginosa* (mm), ampicillin not active[b]	14.1±0.5	17.0±0.6	16.0±0.4	17.2±0.3	16.3±0.6
Zones of inhibition *E. coli* (mm), ampicillin, 15.2±0.7 mm[b]	12.1±0.5	15.0±0.5	12.6±0.5	13.1±0.4	17.9±0.8
Zones of inhibition *S. enteritidis* (mm), ampicillin, 17.0±0.6 mm[b]	13.0±0.6	15.2±0.3	11.0±0.1	17.8±0.1	14.3±0.4
Zones of inhibition *K. pneumoniae* (mm), ampicillin, 16.1±0.1 mm[b]	11.3±0.1	15.0±0.2	9.7±0.6	18.7±0.5	10.6±0.1
Zones of inhibition *S. aureus*, (mm), ampicillin 16.2±0.3 mm[b]	11.7±0.9	15.2±0.3	7.8±0.1	14.3±0.5	not active
Zones of inhibition *C. albicans* (mm), nystatine 18.7±0.2 mm[b]	13.1±0.1	14.0±0.5	12.9±0.2	14.3±0.6	13.0±0.2
Zones of inhibition *A. niger* (mm), nystatine 17.2±0.5 mm[b]	14.0±0.6	14.2±0.3	12.6±0.2	15.3±0.6	12.2±0.3
Incidence of micronuclei formation per 1,000 cell[b]	44.9±1.3	48.1±0.4	44.9±1.2	57.3±1.9	48.3±1.2
Incidence of micronuclei formation relative to the incidence of micronuclei formation in the control sample (%)[b]	21	30	21	54	30

[a] Mean value of three experiments
[b] Mean value of five measurements±SD; Growth inhibition zone including disk diameter, 6 mm (5 μg of extract per disk); ampicillin and nystatine (30 μg per disk); Incidence of micronuclei formation in the control sample per 1,000 cell; 37.2±1.8

millimeters using a "Fisher-Lilly Antibiotic Zone Reader" (Fisher Scientific Co. USA). Each test was performed in quintuplicate. Mean values were presented.

Micronucleus Test

This assay was carried out according to the known procedure [6]. The blood samples were obtained from 5 healthy nonsmoking male donors (average age—42.3 years). All five samples were treated in the following way: blood samples (0.5 ml,) were added to 5 ml of RPMI-1640 medium supplemented with 15% calf serum and phytohemaglutinin 2.5 mg/ml (Sigma, St. Louis, USA). One hour after initiating the cell stimulation, the extracts of *Equisetum* sp. (at final concentrations of 62.5 μg of the extracts per solution ml) or quercetin (at final concentrations at 1.3 μg of the extracts per solution ml) or kaempferol (at final concentrations at 1.2 μg of the extracts per solution ml) were added to the cultures. The cultures without extracts were used as a negative control. All cultures were incubated at 37°C for 19 h, then washed with the medium, transferred into 5 ml of fresh medium and incubated for a further 48 h. Cytochalasin B (at a final concentration of 6 μg/ml) was added to the cultures and the samples were incubated for 24 h. The cells were collected by centrifugation at 1,200 rpm, washed with 0.9% NaCl, then with hypotonic solution. The cell suspension was prefixed in methanol/acetic acid 3:1 (*v*/*v*), washed three times with the same solution, and dropped onto a clean slide. The slides were air dried and stained with alkaline Giemsa (2%), 1,000 cells per sample were analyzed with an Axiphot-2 fluorescence microscope (Carl Zeiss, Jena, Germany). The results are given in Table 2. as the number of micronuclei containing cells per 1,000 scored cells and as incidence of micronuclei formation relative to the incidence of micronuclei formation in the control sample.

Statistical Analysis

In order to evaluate statistically any significant differences among mean values, a one-way ANOVA test was used. In all tests the significance level at which we evaluated critical values differences was 5%.

Results and Discussion

The results of the performed assays are summed up in Tables 1 and 2.

HPLC Profiles

Contrary to the work of Veit et al. [11], where quercetin 3-*O*-(6″-*O*-malonylglucoside) was reported to be the main component (28–50%), we found the non-malonylated quercetin

3-*O*-glucoside (49.6%) and the free aglycone quercetin (21.1%) as the major constituents of the *E. arvense* hydro-alcoholic extract (20:80, *v/v*). Making the result even more interesting is the fact that in the same paper, Veit et al. [11] determined that the relative amount the non-malonylated derivative never exceeds the content of quercetin 3-*O*-(6″-*O*-malonylglucoside) throughout the vegetation period. However, they also noticed that during the growth period of *E. arvense*, the amount of quercetin 3-*O*-(6″-*O*-malonylglucoside) decreases with the simultaneous increase of the non-malonylated derivative. One possible explanation for the high content of quercetin and the unesterified glucosides in our samples could be the spontaneous hydrolysis (cleavage of the glycoside and ester bonds) during the extract preparation procedure or/and HPLC analyses. But, it is also possible that the aforementioned compounds are present in the dry plant material since, in the samples of the other four *Equisetum* species, treated in the same way, the aglycones were not detected at all or found to be only minor contributors (Table 1). The difference in the extraction procedure (boiling methanol [11] and room temperature extraction [this work]) also favors the view of the non-artefactual origin of the aglicons and non-malonylated derivatives.

All of the samples (Table 1) contain caffeic acid conjugates, compounds characteristic for the early stages of phenological development of *Equisetum* sp. [11]. These depsides are presumed to defend the plants against pathogens until the accumulation of silica in the epidermal cells is not sufficient to take over the role of plant primary protection [11].

The main components in *E. sylvaticum* extract were found to be quercetin 3-*O*-glucoside (44.7%) and kaempferol 3-*O*-glucoside-7-*O*-rhamnoside (12.5%). The malonylated derivatives of the above mentioned compounds were not detected in our samples although they were found to be major constituents in Veit's et al. [11] samples of *E. sylvaticum*. A possible cause of this discrepancy may be the chemotypification of this species, or less likely, as previously discussed, the hydrolysis of the ester bonds during the extraction and/or HPLC analyses and/or the application of a different extraction method.

The composition of *E. fluviatile* extract was not as complex as the other extracts with kaempherol 3,7-*O*-diglucoside as the main component (47.6%) (caffeic acid conjugates were also major contributors, in total 29.4%). The HPLC profile of *E. telmateia* extract (kaempferol 3,7-*O*-diglucoside—23.6% and caffeic acid conjugates in total 20.6%), was similar to *E. fluviatile* profile, however, was additionally characterized by a relatively high content of acetylated flavonoid derivatives, kaempferol-3-*O*-(6″-*O*-acetylglucoside) (10.4%), kaempferol 3-*O*-(6″-*O*-acetylglucoside)-7-*O*-glucoside (12.7%) and kaempferol 3-*O*-(6″-*O*-acetylglucoside)-7-*O*-rhamnoside (10.1%) (so far, in the genus *Equisetum*, found only in *E. telmateia*). The acetylated derivatives were present in higher amounts compared to the non-acetylated ones, except in the case of kaempferol 3,7-*O*-diglucoside (23.6%) and kaempferol 3-*O*-(6″-*O*-acetylglucoside)-7-*O*-glucoside where the ratio was reversed. In Veit's et al. [11] *E. telmateia* samples, kaempferol 3-*O*-(6″-*O*-acetylglucoside) was not detected, and in a recently published paper [8], kaempferol 3,7-*O*-diglucoside was not reported as the *E. telmateia* extract constituent.

E. palustre extract consisted mostly of kaempferol glycosides–kaempferol 3-*O*-rutinoside-7-*O*-sophoroside (24.7%), kaempferol 3-*O*-rutinoside-7-*O*-glucoside (12.3%) and kaempferol 3-*O*-glucoside (11.9%). This result stands in good agreement with Veit's et al. [11] work, although, kaempferol 3,7-*O*-diglucoside was not detected in our samples even though it was one of the main components noted in the work of Veit et al. [11].

Total Phenol Content

Both the extract yields and the phenol contents (Table 2) follow the same trend of increasing values: *E. arvense*, *E. fluviatile*, *E. palustre*, *E. telmateia* and *E. sylvaticum*. The ratio between the content of total phenols and the yield of extracts is roughly 10 μmol/% (from 15 for *E. sylvaticum* to 10 for *E. arvense* and *E. palustre*). The obtained values for the total phenol content in the dry plant material (92-349 μmol per gram of dried plant material) are considerably higher than those previously published for Equiseti herba (60 μmol of catechin equivalents per g [7]), while the total phenol content of *E. telmateia* (infusion) was comparable to our results (ca. 460 μmol of gallic acid equivalents per g of dried plant material [8]). Expressed as the number of μmol of chlorogenic acid per g of the dry extract, the total phenol content ranges from 1,061 μmol/g for *E. palustre* to 1,531 μmol/g for *E. sylvaticum*. These values are much higher than those previously reported for *E. maximum* (320 μmol/g) [14] and two fully unidentified *Equisetum* sp. (200 μmol/g and 744 μmol/g) [15] tested in an analogous assay.

Antioxidant Capacity

As clearly evident from Table 2, the values of the antioxidant capacity varied quite markedly, from very low determined for *E. palustre* and *E. fluviatile* to high values obtained for *E. arvense* and *E. telmateia*. Although the phenol content was shown to be the greatest in the extract of *E. sylvaticum* (Table 2), the antioxidant capacity of that extract turned out to be only average among the tested samples. Thus, it can be concluded that our results do not

confirm the correlation between the total phenol content and the antioxidant capacity that was established by Katalinic and co-workers [7] but, having in mind the different HPLC profiles of the extracts, show that the antioxidant capacity depends not only on the total phenol content but also on the specific phenol composition. The total number of phenolic hydroxyl groups as well as their position on the aromatic core influences the antioxidant capacity—the greater the number of *ortho* or *para* oriented phenolic hydroxyl groups the antioxidant activity enhances due of the inherent stabilization of the formed radicals via delocalization [16]. The high antioxidant activity of *E. arvense* and *E. telmateia* extracts could be attributed to the high content of quercetin and its moiety in quercetin glycosides (*E. arvense*) and caffeic acid derivatives (*E. telmateia*) (both, quercetin and caffeic acid have *ortho* oriented phenolic groups). Even though the percentage of caffeic acid derivatives (29.4%) in *E. fluviatile* extract is higher than the one in the *E. telmateia* extract (20.6%) the antioxidant capacity is ca. 75 times lower than the capacity of *E. telmateia* extract. Thus, it seems that the acetylated derivatives showed greater antioxidant activity than the nonesterified ones. *E. sylvaticum* extract contains both kaempferol and quercetin glycosides and shows moderate activity. As expected, *E. palustre* extract with only kaempferol derivates present, exhibited low antioxidant capacity. These results qualify *E. arvense*, *E. telmateia* and *E. sylvaticum* as rich sources of natural antioxidants.

Antimicrobial Activity

The results of the antimicrobial assay showed that the Gram-positive *S. aureus* is the most resistant strain to all examined extracts and these findings seem not to be in accord with the conclusions of previous screenings of medicinal plants for antimicrobial activity, where most of the active plant extracts showed activity against Gram-positive strains [17–19]. The most sensitive strain was *P. aeruginosa* with the average inhibition zones ranging from 14–17 mm (Ampicillin did not affect the growth of the strain at all, Table 2). The activity of *Equisetum* extracts against two fungal organisms (*C. albicans* and *A. niger*) was lower than that of Nystatine. *E. palustre* and *E. sylvaticum* extracts showed stronger activity than other extracts (this parallels the identity of the sugar components of major flavonoid glycosides, rhamnose, rutinose and sophrose in *E. palustre* and *E. sylvaticum* extracts, while the remaining three extracts contained glucose as the sugar component). Generally speaking, the antimicrobial activity of the tested *Equisetum* extracts in the dose of 5 μg of the extract per disk was comparable to the activity of the positive controls (Ampicillin and Nystatine, 30 μg per disk) and these could then be considered as good candidates for raw material antimicrobial phyto-preparations.

Micronucleus Test

All tested extracts (62.5 μg/ml) showed higher incidence of micronucleus formation than that of the control sample (cytochalasin B, 6 μg/ml), and expressed as percentages relative to the control, these values ranged from 21 to 54%. The incidence of micronucleus formation caused by *E. arvense* and *E. fluviatile* is comparable with that caused by quercetin alone (ca. 20% at a concentration of 1.3 μg/ml), while that of *E. telmateia* and *E. sylvaticum* with the effect of kaempferol (ca. 30% at a concentration 1.2 μg/ml). *E. arvense* extract contains only quercetin derivatives, while *E. fluviatile* extract only kaempferol derivatives. A previous study reported that the incidence of micronucleus formation in Equiseti herba extract treated cells was concentration dependent [6], and at a concentration of 200 μg/ml, was three times higher than that in the control. *E. palustre* extract showed the greatest effect, since its phenol content and antioxidant capacity is the lowest of all examined extracts. Hence, it seems that the usual mechanism, by the reactive oxygen and nitrogen radical number reduction, causing the decrease in the level of induced DNA damage, is operative in the case of examined *Equisetum* extracts.

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