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Phytochemical analysis and *in vitro* assessment of *Polystichum setiferum* extracts for their cytotoxic and antimicrobial activities

Nicoleta Anca Şuţan[1,*], Irina Fierăscu[2], Radu Fierăscu[2], Deliu Ionica[1], Liliana Cristina Soare[1]

[1] *University of Piteşti, Faculty of Sciences, Physical Education and Informatics, Department of Natural Sciences, 1 Targu din Vale Str., 110040 Pitesti, Romania*
[2] *The National Institute for Chemistry and Petrochemistry – ICECHIM, 202 Spl. Independentei, 060021, Bucharest, Romania*
*Corresponding author: ancasutan@yahoo.com

Abstract. Ferns are traditionally used by some nations to treat rheumatism, lungs, gynecology, blood and digestion dysfunctions, and several others illnesses. The present study evaluates the bioactivity of methanol and ethanol extracts from *Polystichum setiferum* (Forssk.) Moore ex Woyn. in an *Allium cepa* test and disk diffusion test. In the *Allium cepa* test the methanol and ethanol extracts induced a significant time-related increase in the mitotic index. The tested extracts were non-mutagenic by used assay, with no occurrence either the structural or numerical aberrations detected. The extracts were also evaluated in terms of trace elements (by EDXRF) and qualitative composition (by UV-VIS, FTIR and total phenolic content). In the disk diffusion test, methanol extracts from leaves determined a small inhibition of bacterial growth for *Enterobacter cloacae* and *Citrobacter freundii* strains relatively to control sample (methanol). The ethanol extracts were more efficient, the diameter of inhibition growth zones measured from 7 to 10 mm, the most affected strain was *Chryseobacterium meningosepticum*.

Keywords. *Polystichum*, extracts, FTIR, EDXRF, UV-VIS, cytogenotoxicity, antimicrobial activity.

INTRODUCTION

Appearance of antibiotic microbial resistance is present all over the world and is an increasingly serious threat to global public health. From the developing countries to the developed ones, bacterial antibiotic resistance problem it is very serious because cases of infection are treated by the lack of medicines or using them in excess (Mundy et al. 2016). Nosocomial infections usually represent an infrequent phenomenon. If it most developed countries this phenomenon is controlled, in many developing countries it is not reported (Serban et al. 2012).

In order to obtain alternative ways to combat bacteria that cause infections, herbal medicine represents one of the most important fields of tradi-

tional medicine all over the world, special attention has being paid to natural extracts with potential antimicrobial biological active compounds (Chanda et al. 2011; Jeyaseelan et al. 2012; Chanda et al. 2013; Fierascu et al. 2015).

The natural products have been considered as a source of potential medicines, specific for each region or country (Dubey and Padhy 2013; Panahi et al. 2014; Gyawali and Ibrahim 2014; Georgiev 2014; Schnekenburger et al. 2014; Atanasov et al. 2015).

In this context the study of ferns can be beneficial for this domain. Studies focused on some ferns used as crude extracts, standardized extracts, purified substances or in the form of nanoparticles showed pharmacological activity (Santos et al. 2010; Soare and Sutan 2018). The total flavonoid contents, antioxidant and anticancer activities, and acetylcholinesterase (AChE) inhibition potential of fern extracts were investigated in detail (Xia et al. 2014), but not the antimicrobial effect and cytotoxicity.

Some ferns species were traditionally used for scabies, eczema, jaundice including wounds and skin disease (Kirtikar and Basu 1999). *Polystichum* is one of the most diverse genera of ferns with 360–400 species distributed worldwide (Morero et al. 2015). *Polystichum setiferum* (Forssk.) Moore ex Woyn. is an evergreen or semi-evergreen fern native to southern and western Europe. Of the four species of *Polystichum* present in Romania, *Polystichum setiferum* (Forssk.) Moore ex Woyn. is the most common species (Sârbu et al. 2013). Totally, one hundred fern species have been described for their ethnomedicinal applications and chemical constituents in a fern ethnomedicinal plant database (Thakar et al. 2015). Rhizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn. tied around the neck of child are used to cure dysentery during the primary teeth development (Kumar et al. 2013), parts of *Polystichum pungens* (Kaulf.) C. Presl are used for the treatment of wounds (Grierson and Afolayan 1999), aerial parts of *Polystichum munitum* (Kaulf.) C. Presl are used to stimulate digestion (Lans et al. 2007), decoctions obtained from the rhizomes of *Polystichum pungens* (Kaulf.) C. Presl Roth are used to treat intestinal worms and as a general anthelminthic, powdered dried fronds to heal wounds, and fresh fronds as poultice (Lall and Kishore 2014), sporophyll extracts of *Polystichum squarrosum* (D. Don) Fee and *Polystichum moluscens* (Bl.) T. Moore are used as antibacterial agent (Singh 1999), decoction obtained from fronds of *Polystichum woronowii* Fomin is used as antiinflamatory and anti-hepatitis agent and decoction obtained from leaf of *Polystichum aculeatum* (L.) Schott as anthelminthic (Bahadori et al. 2015).

The present study aims to comparative analyse the chemical composition of the methanol and ethanol extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn., their antimicrobial effects on five bacterial strains: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterobacter cloacae* and *Citrobacter freundii* (both clinical strains) and *Elizabethkingia meningoseptica* (formerly *Chryseobacterium meningosepticum*, strain isolated from soil) and their cytotoxic effects on meritematic root cells of *Allium cepa* L. assay.

MATERIALS AND METHODS

Preparation of plant extracts

Leaves and rhizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn. were collected from Vîlsan Valley, Argeş County, Romania from site N 45º25'40.2", E 024º42'33.1", altitude 1062 m. Part of the plant material is preserved (-18 ºC) in Botanic Laboratory of the Department of Natural Sciences, University of Pitesti for future references. The voucher specimen is register at the Botanical Collection of County Museum Argeş with number 11326/25.05.2016. The leaves and rhizomes with scales were washed thoroughly and rinsed with bidistilled water and preserved at -18 ºC. Frozen leaves and rhizomes were chopped at room temperature. Leaves and rhizomes ethanol and methanol extracts (PEL - leaves ethanol extract, PER - rhizomes ethanol extract, PML - leaves methanol extract, PMR - rhizomes methanol extract) were prepared by mixing 100 g of each type of vegetable material in 1000 ml alcohol for 48 h at room temperature (22°C). The obtained extracts were filtered using Whatman filter paper no. 1.

The dried weight was obtained by shade drying to constant mass the plant materials, in order to remove excess moisture. The average dried masses for 15 samples were 29.01%±2.80 for rhizomes and, respectively, 32.25%±2.66 for leaves.

Evaluation of chemical composition of leaf and rhizome extracts

For Fourier transform infrared spectroscopy (FTIR), was used a Varian 3100 Excalibur spectrometer equipped with a Harrick Praying Mantis diffuse reflectance (DRIFT) accessory. The IR spectrum was collected in the region 4000–500 cm-1 at a resolution of 2 cm-1. The spectrum was analysed with the program Analyzer IR – KnowItAll (Bio-Rad 2005).

For UV-Vis evaluation was used an UV - VIS Unicam Helios α Thermo Orion spectrometer from 200 to 900 nm, at the resolution of 1 nm, with 1 nm slit width and automatic scan rate. The obtained results were processed using specific data analysis software (Origin Pro 8.0).

For energy dispersive X-ray fluorescence determinations was used a PW4025 – MiniPal – PANalytical energy dispersive XRF Spectrometer with rhodium anode. The XRF determinations have been carried out in Helium atmosphere, for a period of 300 seconds, without any filter, at proper voltage and current intensity.

Determination of total phenolic contents

The concentration of total phenolic was measured by colorimetric method with Folin- Ciocalteu reagent (Merck KGaA Germany), according with a method previously presented (Fierascu et al. 2015). The method involves the reduction of Folin-Ciocalteu reagent by phenolic compounds, with formation of a blue complex; the absorbance was read at 765 nm on the UV-VIS spectrophotometer. The measurements were compared to a standard curve prepared with gallic acid (99%, Merck KGaA Germany) solutions at different concentrations (10-55 µg/mL). The total phenolic content was expressed as milligrams of gallic acid equivalents per 100 gram of dried weight. The dried weight was obtained by shade drying to constant mass the plant materials, in order to remove excess moisture.

Antioxidant activity

Antioxidant activity of the extracts was determined following the DPPH assay, following a protocol previously exhaustively described (Fierascu et al. 2015, Fierascu et al. 2014). The absorbance was read at 517 nm after an incubation period of 30 minutes. The antioxidant activity (AA %) percentage was calculated using the formula:

$$AA\ (\%) = [(A_{control}\text{-}A_{sample})/A\_control]\times 100,$$

where: $A_{control}$ is the absorbance of the DPPH solution without sample, A_{sample} is the absorbance of the extract mixed with 0.02 mg/mL DPPH solution.

Evaluation of antimicrobial properties

Antibacterial effects of fern methanolic and ethanolic extracts were tested by disk diffusion method on five bacterial strains: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterobacter cloacae* and *Citrobacter freundii* (both clinical strains) and *Elizabethkingia meningoseptica* (formerly *Chryseobacterium meningosepticum*, strain isolated from soil). Frequency and rank of isolated bacteria, identified and tested by antibiogram in microbiology laboratories within major hospitals in Bucharest present these strains in the top of the isolated germs that produce nosocomial infections (Serban et al. 2012).

As positive control was used antibiotic (Ampicillin 10µg per disc from Bioanalyse), while the negative control was the solvent specific for each extract (ethanol 96% and methanol 99.8%).

An overnight (16 to 24 h) culture at 37°C of each bacterial strain was used (bacterial suspension was prepared by suspending one wire loop from the stock into 2 mL nutrient broth).

In disk diffusion method sterile filter paper discs with 6 mm diameter were used. For diffusion method was used nutrient agar in Petri dishes. Each bacterial culture was homogeneous inoculated (in three directions) on the entire surface of nutrient agar in Petri dishes.

The disc with Ampicillin (pre-warmed to room temperature) was placed on the surface of inoculated solid medium. Four discs of sterilized filter paper were put on the nutrient agar; 5µL of each fern extract were used to impregnate the discs using micropipette. For negative control, two sterile discs of paper filter were used (5µL ethanol, respectively 5µL methanol). The Petri dishes were incubated inverted for 24 h at 37°C.

The antibacterial effects of fern extracts were estimated by measuring the diameter of inhibition growth zone (in millimeters), as a clear zones surrounding paper filter discs. The experiment was repeated three times and the results were in terms of average of measured values.

Evaluation of cytotoxic activity of leaf and rhizome extracts

Cytotoxic potential of leaf and rhizome extracts was evaluated by changes in mitotic index (MI) and phase indexes (prophase, metaphase, anaphase, telophase), induced in root tips cells of *Allium cepa* L. (Sutan et al. 2016). Equal-sized onion bulbs, from a local variety, were purchased from local market. The outer scales were carefully removed and the bottoms were scraped to expose root primordia. Rhizogenesis and root growth were induced on 30 ml jars filled with distilled water until to 0.5-1cm average length of the roots. After 48 hours, freshly emerged roots were treated with leaf and rhizome extracts for 6, 12 and 24 hours. Distilled water, ethanol and metha-

nol were used as controls. Cytological analysis were performed on squash slides prepared as follow: the root were fixed in a mixture of ethanol + glacial acetic acid (3:1) for 12 hours at 4°C, than were transferred to a watch glass in preheated 1N HCl at 60°C for 14 minutes and subsequently were immersed in preheated aceto-orcein solution at 60°C for 14 minutes. The tips of the roots were cut on a glass slide in a drop of 45% acetic acid, covered with coverglass, and squashed by tapping with matchstick. About 3000 cells from 9 root tips were scored for each treatment. The cells at different stages of mitosis were noticed.

Mitotic index (MI) was computed by determining the mitotic cell frequency (prophase, metaphase, anaphase and telophase) by the total number of cells observed and multiplying the result by 100 (Tedesco and Laughinghouse IV 2012). The number of cells at various mitosis stages (prophase, metaphase, anaphase, telophase) was calculated as percentage to number of dividing cells. Results are presented as the Mean ± standard error of more independent experiments. The data was analysed for statistical significance using analysis of variance (one way ANOVA) and Tukey test was used to determine significant differences among means. Significant differences were set at P≤0.05.

RESULTS

Phytochemical analysis

The mineral content of the extracts was evaluated using a non-destructive technique, X-ray fluorescence. The extracts contain traces (in the ppm concentration range) of Mg, P, Ca, Cr, Mn, Fe, Ni and Cu. Also, PML contains minor traces of K and the ethanol extracts contains traces of Co, not observed for the methanol extracts (Fig. 1A).

The FTIR spectra presented in Fig. 1B showed that the plant have compounds such as aldehyde, ketone, alcohol, carboxylic acid, amides, ethers and phenolic compounds. The peaks in FTIR spectra in Figure 1B are attributed to the following type of compounds, as previously reported (Sutan et al., 2016): hydroxy compounds (OH stretching) – 3649 cm^{-1}, carboxylic acid (OH stretching – 2982 cm^{-1}, 1406 cm^{-1}), carbonyl compounds (C-H stretching – 2901 cm^{-1}, C=O stretching – 1668 cm^{-1}), water (2133 cm^{-1}), allenes (C=C=C bond -1923 cm^{-1}), aromatic ring (1454 cm^{-1}), alcohol (1323 cm^{-1}, 1260 cm^{-1}), aromatic hydrocarbons (880 cm^{-1}) and mineral components (539 cm^{-1}). The peaks at 1067 cm^{-1} and 1040 cm^{1} are attributed to characteristic functional groups of polyflavonoids and, respectively, –C-O- groups of the polyols such as flavones and terpenoids.

UV-VIS spectrum (Fig. 2) of the leaves extract (PML and PEL) seems to be a complex mixture of pigments: chlorophyll *a* – around 415 nm and 660-670 nm; chlorophyll *b* – 450 and 640 nm, and in less extent, carotenoids, while the rhizomes extracts (PMR and PER) contains traces of chlorophyll *a* and minor traces of chlorophyll *b.*

The total phenolic content and antioxidant activity of the extracts (Table 1) shows a direct correlation between the phenolic content and the antioxidant potential.

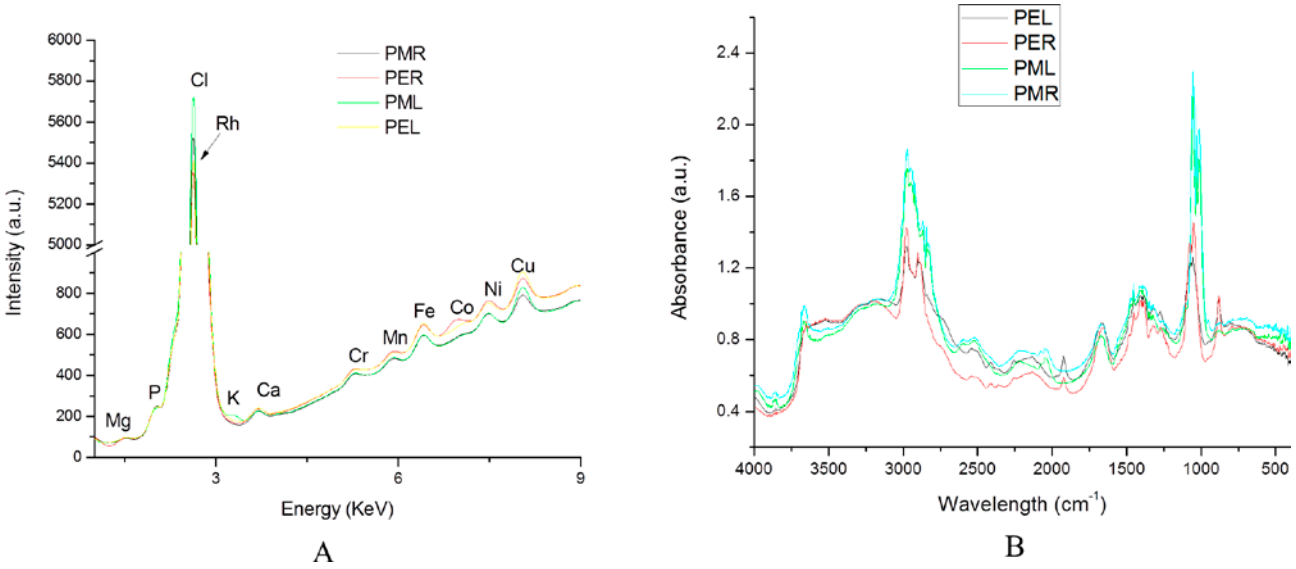

Fig. 1. EDXRF spectra (A) and FTIR spectra (B) of the ethanol and methanol extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn.

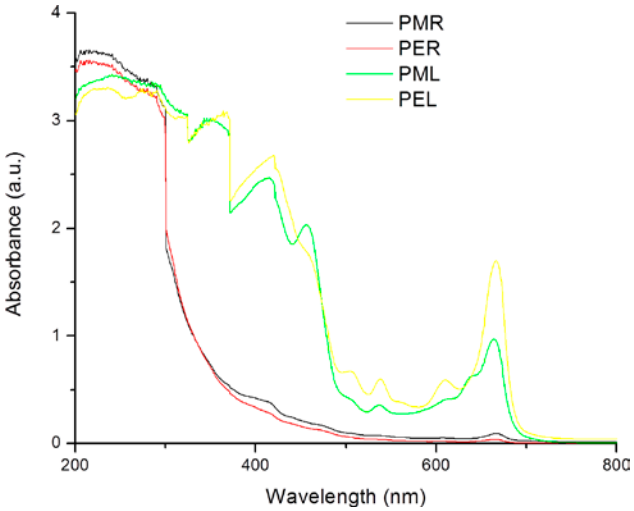

Fig. 2. UV-Vis spectra of the ethanol and methanol extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn.

Table 1. Total phenolic content and antioxidant activity of the ethanol and methanol extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn.

Extract	Total phenolic content (mg GAE/100 g dry mass)	Antioxidant activity (%)
PMR	187.25±0.78	95.58±0.95
PML	44.64±0.16	74.32±0.62
PER	161.24±0.65	92.55±0.68
PEL	23.57±0.12	90.38±0.55

Antimicrobial activity

Antimicrobial activity of fern extracts is illustrated in Table 2. Statistical analysis of experimental data shows that the highest value for inhibition zone (9.5±0.28 mm) was determined for PEL against *Elizabethkingia meningoseptica*. Except *Citrobacter freundii,* the microorganisms being tested displayed significant differences of ≤2 mm between inhibition zone induced by PEL and corresponding concentration of ethanol. In comparison, the smallest inhibition zone was noted for the methanol extracts, irrespective of the part of the plant material used in extraction or the type of microorganisms tested.

Mitotic index variation

The effect of *Polystichum setiferum* (Forssk.) Moore ex Woyn. extracts is relevant, but there is not data about their effect on MI, so the MI of root tips cells to leaves and rhizome extracts was evaluated (Fig. 3). The highest frequency of cells undergoing mitosis was noted in control samples. The MI in meristematic root cells of *Allium cepa* L. treated with *Polystichum setiferum* (Forssk.) Moore ex Woyn. methanol and ethanol extracts of leaves follow similar trends: decreased at the minimum of 6h treatment and rise progressively with increasing of time exposure to 12 and 24h. The MI values ascertained for PEL were slightly lower, when comparing with PML, that may be the consequence of higher concentration of metals in ethanol extracts, as EDXRF analysis demonstrates.

Table 2. Antimicrobial activity of the extracts of leaves and rhizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn. (inhibition zone – in mm).

Extracts/ Control	Tested microorganisms				
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Enterobacter cloacae*	*Citrobacter freundii*	*Elizabethkingia meningoseptica*
	IZ	IZ	IZ	IZ	IZ
PMR	7.00±0.28 fghi	R	6.16±0.16 hi	6.16±0.16 hi	R
PML	7.00±0.00 fghi	R	6.5±0.18 hi	6.83±0.16 ghi	R
PER	8.00±0.00 f	R	7.33±0.33 fgh	9.16±0.16 e	6.66±0.33 ghi
PEL	9.33±0.33 e	8.00±0.00 f	7.66±0.33 fg	7.33±0.33 fgh	9.5±0.28 e
Ampicillin	14±0.00 d	25.66±0.33 c	30.33±0.33 a	9.33±0.33 e	29±1.00 b
Ethanol	7.33±0.33 fgh	6.16±0.16 hi	6.66±0.33 hi	7.66±0.33 fg	7.33±0.88 fgh
Methanol	7.00±0.57 fghi	6±0.00 i	6.66±0.57 hi	6.33±0.57 hi	6.33±0.57 hi

*Means with the same letter are not significantly different from each other (Tukey test, P>0.05)
* R – resistant

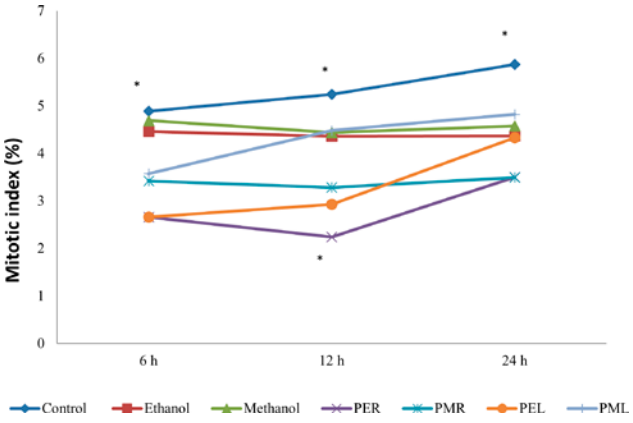

Fig. 3. The influence of the extracts of leaves and rhizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn. on the mitotic index in meristematic root cells of *Allium cepa* L. (*the interpretation of the significance of the differences by means of the Tukey test, p<0.05).

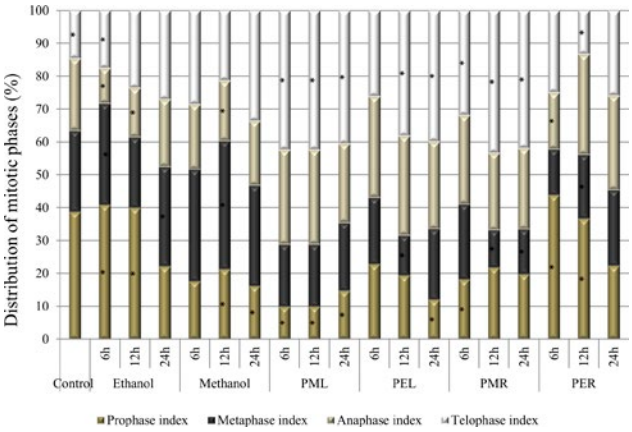

Fig. 4. The influence of the alcoholic extract of the rhizome and leaves of *Polystichum setiferum* (Forssk.) Moore ex Woyn. on the distribution of the mitotic phases in the meristematic root cells of *Allium cepa* L. (*interpretation of the significance of the differences, by means of the Tukey test, p<0.05).

Distribution of mitotic phases

Ethanol and methanol extracts affected the percentage of mitotic phases for all tested times (Fig. 4). We showed here that treatments with methanol extracts of leaves and rizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn., except PML-24h, had caused mitotic arrest in meristematic root cells of *Allium cepa* L., accumulating telophase cells. Prophase cells frequency decreased significantly in these experimental conditions. In contrast, the frequency of telophase cells decreased in root tips treated with ethanol extracts, except PEL-12h and PEL-24h.

DISCUSSION

As a general remark, the ethanol seems to extract with higher efficiency the metals from the samples, as all the metals are in higher concentration in the ethanol extracts, while P has the same concentration in all the samples. It can be observed that the methanol extracts have higher intensity of the absorbance bands, which can be correlated with the results obtained for the total phenolic content and with the UV-VIS analysis.

It can be noticed that all extracts presents strong absorption bands corresponding to phenolic acids (more intense for the rhizomes extracts). Also, even if the methanol seems to be a more efficient solvent for the extraction of the phenolic compounds, the ethanol seems to be more efficient for the extraction of pigments.

Antioxidant activity has been noticed for other species of *Polystichum* genera, such as *Polystichum lepidocaulon* (Hooker) J. Smith, *Polystichum polyblepharum* (Roem ex. Kunze) C. Presl (Shin 2010) and *Polystichum semifertile* (C.B. Clarke) Ching (Ding et al. 2008). According to Shin (2010) the crude extracts obtained from some ferns, such as those of the genera *Davallia*, *Hypolepis*, *Pteridium*, *Cyrtomium*, *Dryopteris*, *Polystichum*, *Dicranopteris*, *Lycopodium*, *Osmunda*, *Adiantum*, *Coniogramme*, *Polypodium*, *Pyrrosia*, *Pteris*, *Lygodium*, *Selaginella*, *Thelypteris*, *Athyrium*, *Matteuccia*, *Onoclea* şi *Woodsia* have strong antioxidant properties, sometimes substantially more effective than other natural or synthetic antioxidants.

Statistical analysis of experimental data indicates that PEL had a significant antimicrobial activity against gram negative bacteria, *Escherichia coli* and *Elizabethkingia meningoseptica;* furthermore, PEL was solely responsible for any antimicrobial activity noticed against *Staphylococcus aureus*, as comparing with PER, PML and PMR. Aerobic *Gram*-negative bacilli *Citrobacter freundii* showed statistic significant sensitivity to PER.

The antimicrobial assay shows no correlation between the phenolic content and the antimicrobial potential. Thus, the antimicrobial effect observed might be assigned to the other type of compounds identified by FTIR, as well as to the metals identified by X-ray fluorescence in higher concentration in the ethanol extracts. Recent studies indicate that different metals cause various types of injuries to microbial cells as a result of generation of reactive oxygen species (ROS), membrane damage, interruption of electron transport, protein dysfunction or DNA damage and inhibition of DNA replication (Lemire et al. 2013; Dizaj et al. 2014).

Antibacterial properties of extracts obtained from leaves of a various fern species, such as *Asplenium nidus*,

Blechnum orientale, C*ibotium barometz*, *Dicranopteris linearis* var. *linearis*, against Gram positive bacteria (e.g. *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, as well as Gram negative bacteria (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* (formely *Salmonella choleraesuis*), *Enterobacter aerogenes*, *Klebsiella pneumoniae*) have been noticed by Lai et al. (2009). These results justify and certify the use of these species in traditional medicine.

The *Allium* root tip biossay was widely applied as bioindicator of pesticides, fertilizers and heavy metals cytogenotoxic effects, but is especially relevant for evaluation of the bioactivity of plant extracts (Bonciu et al., 2018).

Taking a comparative approach to the cytogenetic effects of extracts of leaves and rhizome, respectivelly, methanol and ethanol extracts of rhizome have had a stronger mitodepressive effect over the meristematic root cells. The decrease of MI in the root tips of *A. cepa* L. has already been highlighted as indicator of the antiproliferative activity of different extracts, such as Frescura et al. (2012) evaluating the *Luehea divaricata* extracts, Kuhn et al. (2015) who studied the leaves and fruits of *Eugenia uniflora* infusions, Sutan et al (2018) assessing the *Aconitum toxicum* Reichenb. rhizome extracts. The MI of samples that has been treated with PER for 12h was significantly lower than in the control.

The higher values of MI determined in the root meristems treated with methanol extracts may be directed related to the higher content of phenolic acids (fig. 1B). Due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors (Amarowicz et al. 2010), phenolic compounds exert protective effect against damaged mediated by ROS.

As the oxidative stress exerts its effect on the cell cycle shortly after the stress was imposed (Reichheld et al. 1999, cited by West et al. 2014), this variation may be the consequence of stress adaptation that prevents cell death or constitute a radical route to cell death. Results presented by West et al. (2014) regarding the response of primary root of *Arabidopsis* to salt stress suggested that plants have a general mechanism that rapidly blocks cell cycle progression under stress conditions, presumably to impede transition to a stage where the cells are susceptible to damage (e.g. M-phase), simultaneously the cellular defence system being activated. Stress-adapted cells undergo cell cycle stages at default rates.

The root tips incubated in alcoholic extracts of leaves and rhizome have revealed these variations of MI values, without showing any chromosome breaking action.

Comparing to ethanol and methanol effects on mitosis in onion root tips, mitotic inhibition induced by extracts of leaves and rhizomes of *Polystichum setiferum* (Forssk.) Moore ex Woyn. was more intense. Decreasing and significant decreasing of mitotic index in root meristems of *Allium cepa* L. suggest the mitodepressive potential of alcoholic extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn., although mitotic delay induced by low concentration of ethylic alcohol were previously noticed by Ancara and Nuti Ronchi (1967).

Increased frequency of telophase cells may be the consequence of high activity of Cdk/mitotic cyclin complex which inhibits the pathway that promotes exit from mitosis. Mitosis progress requires the ubiquitination of proteins whose proteolysis is necessary for chromatid separation and pre-replication complexes assembles, so that the cell is ready to begin DNA replication at the next S phase. When ubiquitination of proteins is inhibited, telophase arrest is induced (Searle and Sanchez 2007).

ROS interference with nuclear envelope dynamics was evidenced by the delayed breakdown of the nuclear envelope at late prophase and its delayed reconstitution at telophase (Livanos et al. 2012), which lead to delayed cell exit from telophase. This delay may be due to experimental disturbance of ROS homeostasis, thus affecting microtubule dynamics and organization (Livanos et al. 2012). It has also been suggested that arrested telophase cells perish by apoptosis (Swe and Sit 1997). Mitotic and chromosomal abnormalities were detected at insignificant levels comparing with controls.

CONCLUSION

The chemical analyses conducted showed a direct correlation between the solvent used for extraction and the total phenolic content. The rhizomes extracts showed a good antioxidant potential, also in good correlation with the total phenolic content.

The antibacterial effect of ethanol extract was stronger against bacteria from soil than clinical bacterial strains. Methanol extracts of fern demonstrated some effects on tested bacterial strains, just clinical ones were slight inhibited by these extracts.

Mitoinhibitory effect of leaves and rhizome extracts of *Polystichum setiferum* (Forssk.) Moore ex Woyn. without cytotoxic and clastogenic effects suggest its antimitotic drugs potential. Although this is an important advance in our understanding of extracts effects further researches must be done. Questions like what concentration of extract for what time of treatment repetition should be used for an excellent cellular response, in order to increase the apoptotic index and induce the cell death remained unanswered.

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