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BIOACTIVITY AND GENOTOXICITY OF CENTURIES OLD REMEDY *ASPLENIUM SCOLOPENDRIUM* L.

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ABSTRACT

Despite the fact that *Asplenium scolopendrium* L. is a wide spread fern which has been used as a human remedy for centuries, there are very poor or no data about activity and genotoxicity of *A. scolopendrium* extracts. In this work, vacuum dried water and ethanol extracts of *A. scolopendrium* fronds were tested for their antimicrobial, cytotoxic and genotoxic potential. Antimicrobial activity was evaluated by disk diffusion assay (concentration of extracts 35 mg/ml, 7 mg/ml and 1,4 mg/ml) and there was no inhibition zone for all extracts and for all microorganisms examined (*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*). Cytotoxic and genotoxic potential of extracts (70 mg/ml, 7 mg/ml and 0,7 mg/ml) was tested using cytokinesis-block micronucleus cytome assay in human lymphocyte cultures. Ethanol blocked division of cells in negative control so only water extracts were analyzed. Extracts didn't show genotoxic properties but they showed weak cytotoxic properties. NDI (nuclear division index) decreased with increasing concentration of extracts, but there was no statistical significance when compared to negative control (p=0,055).

Keywords: Antimicrobial; Genotoxic; Cytotoxic; *Asplenium scolopendrium*

INTRODUCTION

Asplenium scolopendrum L. (syn. *Phyllitis scolopendrium* (L.) Newman; *Scolopendrium officinale* Sm) Aspleniaceae is a fern spread in Europe, East Asia, North America[1] and North Africa.[2] This is very common fern in dark and moist forests in Bosnia and Herzegovina.[1, 3, 4] Fronds of this fern are used in traditional medicine of our region (ex Yugoslavia). They are collected in summer when spores are becoming mature.[5] It has been used as a component of the tea for the treatment of wounds, diuretic, astringent, diaphoretic and as a remedy for cough and lung diseases.[1, 3, 5] Today, in Bosnia and Herzegovina, Serbia and Montenegro commercially available tincture of *Asplenium scolopendrium* can be found. It is marketed with indications: inflammation of gums, gingivitis, stomatitis and parodontal diseases. *A. scolopendrium* is also used in traditional medicine in other parts of the world: in England as ointment for burns and hemorrhoids, decoction or infusion for Bright disease, liver and spleen diseases, and as a mild laxative and tonic.[2, 6, 7] In Romania fronds are used for asthma and cough and roots as astringent and antihaemorrage.[8] In Albania fronds are used for all respiratory and pulmonary diseases.[9] Best view of all indications is presented in Duke et al., 2002 (figure 1).[10] Main active compounds are tannins, choline, vitamin C, essential oils, mucilages, flavonoids (including kaempferol-7-rhamnoside-3-caffeoyl-7-diglucoside), thiaminase (probably present only in the fresh plant), saccharose, invert sugar and terpenoids: lutein, (6S,9S)-roseoside, icariside B_2 and picrionoside A. [2, 3, 5, 11]

MATERIALS AND METHODS

Plant material: Fronds of *A. scolopendrium* L. were collected in September, 2008. on location Ribići, near Konjic, Bosnia and Herzegovina. Voucher specimen (No. 51409) was deposited at the herbarium of National Museum of Bosnia and Herzegovina. Fronds were dried on room temperature in cold, dark and good ventilated place.

Extraction: Dried plant material was powdered and used for extraction. For preparation of dried ethanol extract 10 g of plant material was immersed in 200 ml 70% ethanol, well closed, mixed and put on cold dark place. For five days it was mixed occasionally. After that it was percolated thorough cotton wool, well closed and left two more days on cold dark place. Then it was filtered and vacuum dried. For preparation of dried water extract 60 g of plant material was wetted with 60 ml of water, and then it was overflowed with 1000 ml of boiling water. 30 minutes it was mixed on room temperature and after that it was percolated thorough cotton wool and vacuum dried. Dry extracts were stored in well closed container above silica gel on dry, cold and dark place.

Testing of antimicrobial potential: Three concentrations (35 mg/ml, 7 mg/ml and 1,4 mg/ml) of ethanol and water extracts were examined for antimicrobial potential. Disk diffusion assay was used. Antibacterial potential was tested on two bacterial strains (*Escherichia coli* ATCC 8739 (positive control chloramphenicol (Caesar&Loretz GmbH) and *Staphylococcus aureus* ATCC 6538 (positive control kanamycin (Duchefa, Haarlem, The Netherlands)). Antifungal potential was tested on *Candida albicans* ATCC 10231 with nystatin (Caesar&Loretz GmbH) as positive control. Bacteria were cultivated at Müller-Hinton agar medium (HiMedia Laboratories Pvt. Ltd.) while for cultivation of *C. albicans* Sabouraud Dextrose Agar (Becton, Dickinson and Company Sparks) medium was used. Solvents (70% ethanol and water) were used as negative controls.

Testing of genotoxic and cytotoxic potential Cytotoxic and genotoxic potential of extracts was tested using cytokinesis-block micronucleus cytome assay in human lymphocyte cultures. [12] Blood samples from four persons were used. Cells were treated with vacuum dried extracts dissolved in distilled water or 70% ethanol in three final concentrations (70 mg/ml, 7 mg/ml and 0,7 mg/ml). Negative control was water or 70% ethanol. For each blood sample and tested concentration at least 2000 binucleated (BN) cells were scored in order to determine frequencies of micronuclei (MN), nucleoplasmic bridges (NPB), and nuclear buds. On same slides 500 cells were counted and frequencies of mononuclear, binuclear, trinuclear, and quadrinuclear cells as well as apoptotic and necrotic cells, were calculated. Results were used to estimate effects of extracts on lymphocytes proliferation by calculating the nuclear division index (NDI) and nuclear division cytoxicity index (NDCI). [13, 14] The slides were analyzed at 400x magnification.

Statistical analysis: Data are presented as mean ± standard deviation. Z-test and ANOVA followed by pairwise comparisons with Newmans-Keuls Multiple comparison test were used for more than two group comparisons (Winks 4.5 Professional software (TexaSoft, Cedar Hill, TX, USA) was used). Level of significance was $p \leq 0,05$.

RESULTS AND DISCUSSION

After extraction 13,10% of dried ethanol and 3,63% of dried water extract (when compared to starting dried plant material) was obtained. In agar diffusion test there were no inhibition zones against *S. aureus*, *E. coli* and *C. albicans* for all extracts and all concentrations used. Majd and co-workers obtained similar results. They examined antimicrobial effects of methanol, ethanol and water extracts of underground and aerial parts of *A. scolopendrium.* Water extracts didn't show antimicrobial activity. Methanol and ethanol extracts of underground parts had stronger antimicrobial activity than extracts of aerial parts. *E.coli* was resistant on all extracts. Antimicrobial effects were of bactericidal type. [15] Ethanol blocked division of cells in negative control so only water extracts were analyzed in micronucleus test. BN cells with one and two MN were observed. Results of calculating NDI and NDCI for controls and treated cultures are presented on figures 2 and 3, respectively. Frequencies of MN and NPB are presented on figures 4 and 5, respectively. There was no statistically significant difference between negative control and tested concentrations of extracts in all tested parameters (NDI, NDCI, MN, NPB,). Although extracts showed weak cytotoxic potential (NDI value decreased with increasing concentration of extracts), there was no statistical significance when compared to negative control (p=0,055). Higher concentrations of extracts should be used in order to get more information about cytotoxic potential. Previously it was reported that 10 µg/ml icariside B2 (which can be found in water extract of *A. scolopendrium)* had 19,4% inhibitory rate on hepatoma cancer cell invasion.[16]

CONCLUSIONS

Water and ethanol extracts of *Asplenium scolopendrium* L. frond prepared by using traditional method for preparation of infusum and tinctura didn't show antimicrobic effects. Water extract didn't show genotoxic effects while cytotoxic effects were very weak. Further examination with different extracts and methods is needed to get better insight into bioactivity and genotoxicity of this plant.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

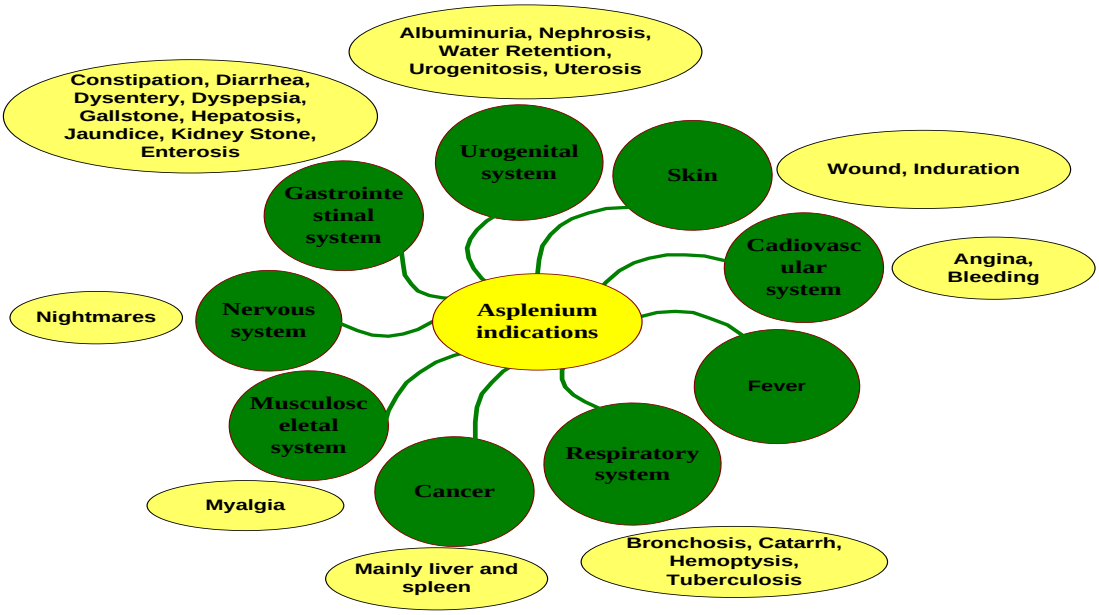

Figure 1: Main indications for *Asplenium scolopendrium* L. (adapted from Duke et al., 2002)

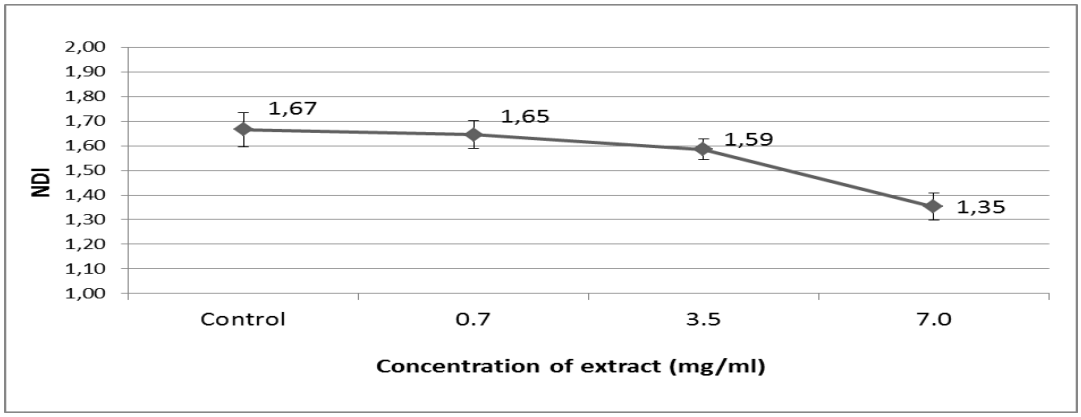

Figure 2: Average values of nuclear division index.

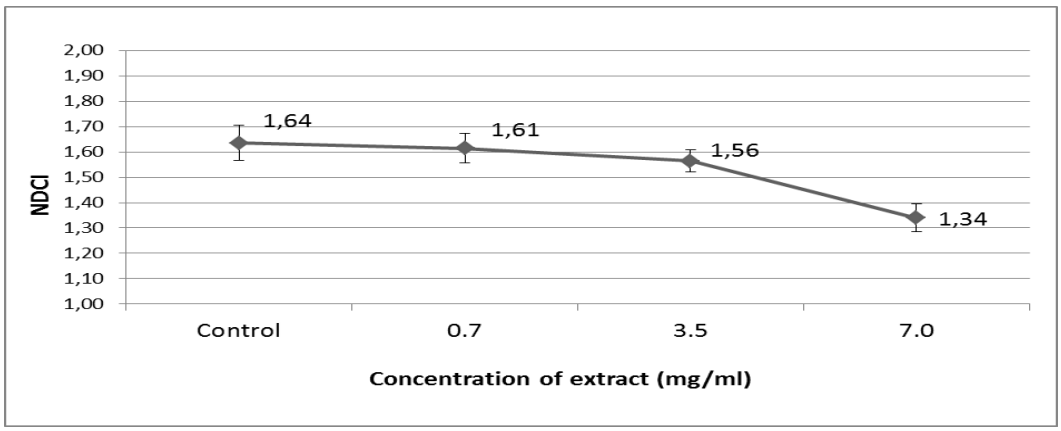

Figure 3: Average values of nuclear division cytoxicity index.

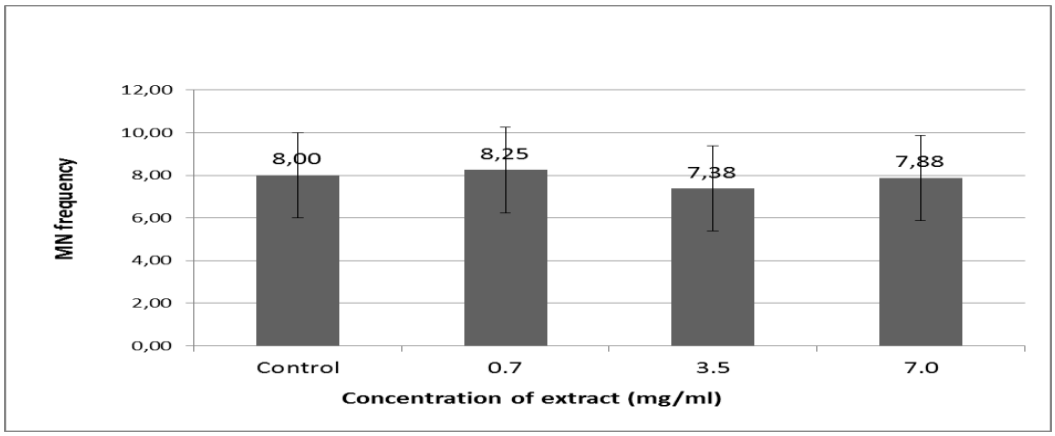

Figure 4: Frequency of micronuclei.

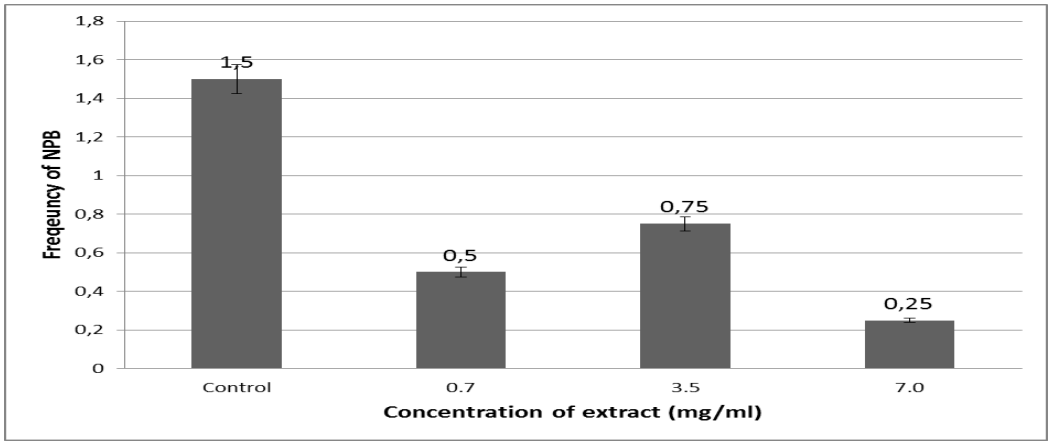

Figure 5: Frequency of nucleopasmatic bridges.

REFERENCES

1. Kušan F. *Asplenium scolopendrium* L. In: Ljekovito i drugo korisno bilje, Zagreb; Poljoprivredni nakladni zavod: 1956, pp. 100.
2. Gruenwald J, Brendler T, Jaenicke C. (eds.). Physicians desk reference for herbal Medicines. 2nd ed., Montvale, NJ; Medical Economics Co: 2000, pp. 381.
3. Tucakov J. Lečenje biljem. Beograd; Rad: 1990, pp. 366.
4. Devetak Z. Ljekovito bilje od znanja do branja. Sarajevo; Šahinpašić: 2004, pp. 188.
5. Tanović N, Saličnović A. Atlas ljekovitog bilja i gljiva Bosne i Hercegovine. Sarajevo; Bemust: 2006, pp. 3.
6. Deschauer T. Illustrated phytotherapy. Book one. Maywood Ill.; Thos. Deschauer Publications: 1945, pp. 24.
7. William L. An Experimental History of Materia Medica. 4th ed., London; J. Johnson: 1791, pp 19.
8. Tita I, Mogosanu GD, Tita MG. Farmacia, 2009; 57 (2): 141-156.
9. Pieroni A, Dibra B, Grishaj G, Grishaj I, Gjon Maçai S. Fitoterapia, 2005; 76 (3-4): 379-399.
10. Duke JA, Bogenschutz-Godwin MJ, duCellier J, Duke KPA (eds.). Handbook of Medical Herbs. 2nd edn., USA; CRC PRESS: 2002, pp. 366.
11. Sohn YM, Chin YW, Yang MH, Kim J. Nat Prod Sci, 2008; 14 (4): 265-268.
12. Fenech M, Nat Protoc, 2007, 2 (5): 1084-1104.
13. Eastmond DA, Tucker JD. Environ Mol Mutagen, 1989; 13 (1): 34–43.
14. Fenech M, Mutat Res, 2000; 455 (1-2): 81–95.
15. Majd A, Mehrabian S, Kelich S, ISAAR 2003, Poster CC02. The fifth Iranian congress of Microbiology. 2003.
16. Ohashi K, Winarno H, Mukai M, Inoue M, Prana MS, Simanjuntak P, Shibuya H. Chem Pharm Bull, 2003; 51 (3): 343-45.