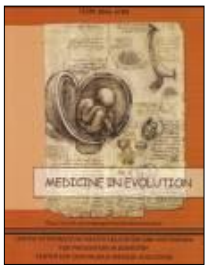
STUDY OVER THE CYTOTOXIC ACTIVITY OF THE POLYGONUM HYDROPIPER L. (POLYGONACEAE)



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ABSTRACT

In order to use Polygonii hydropiperis herba as raw material for the preparation of a pharmacologically active extract, we decided to test the possible cytotoxicity.

Material and methods *– The material used was harvested in August 2009 (Sf. Gheorghe, Tulcea). Species identity and cytotoxicity of the aqueous and ethanolic solutions (Constantinescu method, Triticum test) was verified. The results were evaluated statistically with GraphPadPrism (Kruskal-Wallis and Dunn's tests).*

Results and discussions *– The identity was assessed by comparing with morphological characters found in literature. Triticum test revealed that the inhibitory effect was almost maximum at 5%-1, 66%, partial at 0, 33% and insignificant at 0,033%. Microscopic examination revealed cytotoxicity activity (disorganized nuclear material, nuclei with hypertrophied nucleoli, metaphases in tropokinesis, anaphases and telophases, anaphases with retarded chromosomes).*

Conclusion *– Polygonum hydropiper has shown cytotoxic activity. Because the plant is used traditionally in the treatment of some disorders, we recommend further studies in order to take advantage of its properties.*

Key words*: Polygonum hydropiper L., microscopic, cytotoxicity.*

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INTRODUCTION

Polygonum hydropiper L. sin. Persicaria hydropiper (L.) Spach. (water pepper), Polygonaceae family, is a widespread species in the wetlands of the Northern Hemisphere [1]. Traditionally, this plant is used as a haemostatic, antimicrobial, anthelmintic and anticancer agent [3]. The previous researches have identified various phytochemical compounds, among which we mention: sesquiterpenes (confertifolin, polygondial, warburganal) [4], flavonosides (heterosides of the quercetin, kaempherol, luteolin and apigenine) [4, 5], catechic tannins [4, 6] and sterols (β-sitosterol and stigmasterol) [4]. Due to flavone, tannin and sterol content, based on the correlation between the chemical structure of the active principles and their pharmacological actions, we assume potential hypoglycemic properties.

In the idea of using the product Polygonii hydropiperis herba as a raw material for the preparation of a pharmacologically active extract, we decided to test its cytotoxicity.

MATERIAL AND METHOD

The raw material used in this research was harvested in August 2009 (Sf. Gheorghe, Tulcea County). The identity of the species was verified by macroscopic examination, both on the field and in the laboratory.

Microscopic examination was performed on cross sections of root, stem and leaf (clarified with Javel water and double staining) and on leaf surface preparation (clarified with 5% sodium hydroxide). Microscopic preparations were analyzed on a Nikon Labophot 2 microscope (oc. 10x, ob. 4x, 10x, 40x).

Cytotoxic potential was investigated by Constantinescu method (*Triticum* test). The method involves determining the maximum dilution of the extractive solutions wich, depending on the duration, can influence root elongation and and the karyokinetic film [7]. The research was conducted on roots of wheat germ (*Triticum vulgare* Mill, race Dropia obtained from the Agricultural Research Fundulea). Solutions for analysis were obtained by refluxing for 30 minutes two samples of 2.5 g plant each in 50 mL distilled water and ethanol 50% (w/w). The aqueous solution was marked with PA5 and ethanolic solution with PE5. From both solutions, PA5 and PE5, several dilutions were made, thus being obtained solutions of 3,33%, 2.5%, 1.66%, 0.33%, 0.033% marked with PA3.33, PA2.5, PA1.66, PA0.33, PA0,033 and respectively PE3.33, PE2.5, PE1.66, PE0.33, PE0.033.

The solutions thus obtained were brought in Petri dishes (d = 50mm) and maintained in contact with 10 germinated wheat caryopses with embryonic root of 1cm for 5 days at 25° C and in the absence of light (in a Sanyo MLR-351H Plant Growth Chamber). A control sample was prepared by substituting extractive solutions with distilled water. During the 5 days the length of embryonic roots was determined by linear method. Changes of karyokinetic film were investigated for each sample after 24 hours. Microscopic preparations were obtained by staining vegetative cone from an embryonic root of each sample with dilute acetic orcein solution. The examination was performed on a Labophot 2 Nikon microscope (ocular 10x, objectives 10x, 40x, 100x).

The inhibitory effect was calculated using Microsoft Excel and statistical

assessment with GraphPad Prism (Kruskal-Wallis and Dunn's statistical tests, 95% confidence interval).

RESULTS AND DISCUSSIONS

The identity was established by comparing with morphological characters found in literature [1, 2].

Microscopic examination of root cross-sections revealed a secondary structure with sclerenchyma fibers in cortex and well-developed secondary wood in central region (Fig. 1, A and B). Stems having both types of structure (primary - fig. 2, A and secondary - fig. 2, B) were observed. The following anatomical elements of stem cross-section were established: multiple layers of angular collenchyma (Fig. 2, A and B), cortical parenchyma with air channels (Fig. 2, A), one layer sclerified pericycle (Fig. 2, A and B) and collateral open vascular bundles (Fig. 2, A and B).

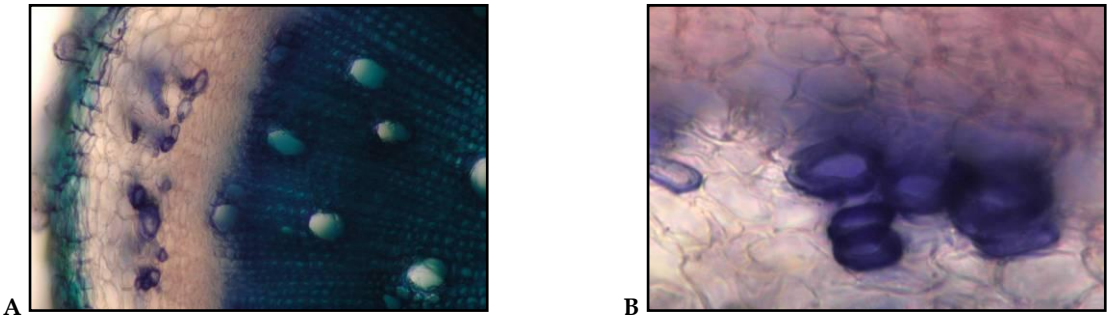

Fig. 1. Root cross-section: A-secondary structure (ob. 10x); B- sclerenchyma fibers (ob. 40x)

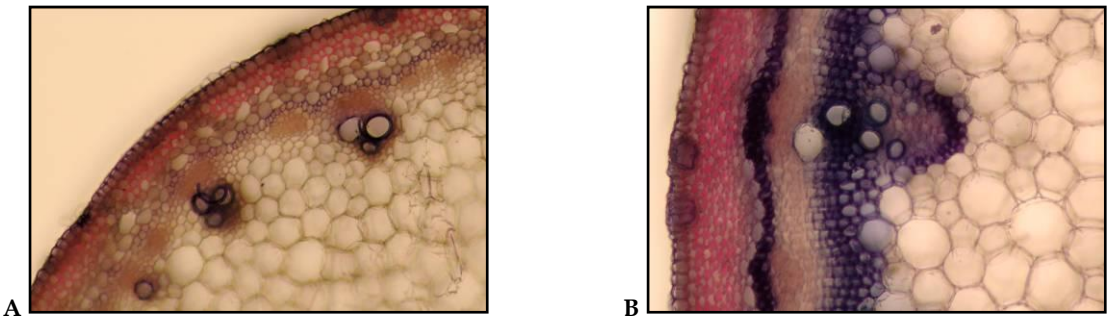

Fig. 2. Stem cross-sections: A-primary structure; B- secondary structure (ob. 10x)

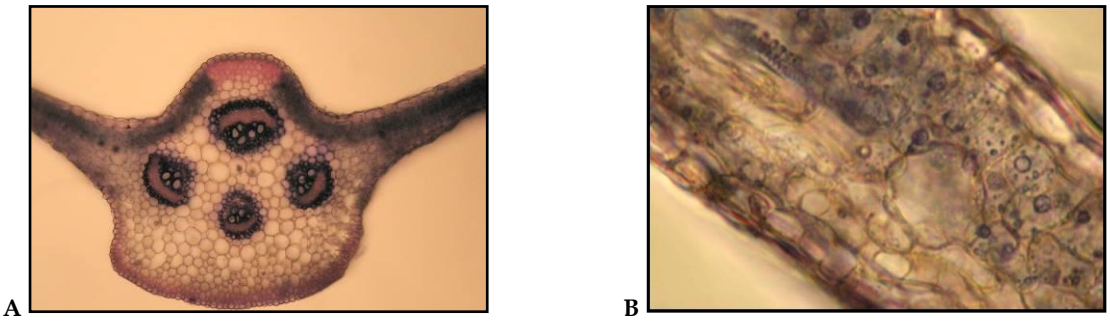

Fig. 3. Leaf cross-sections: A– main rib (ob. 4x); B- leaf blade (ob. 10x)

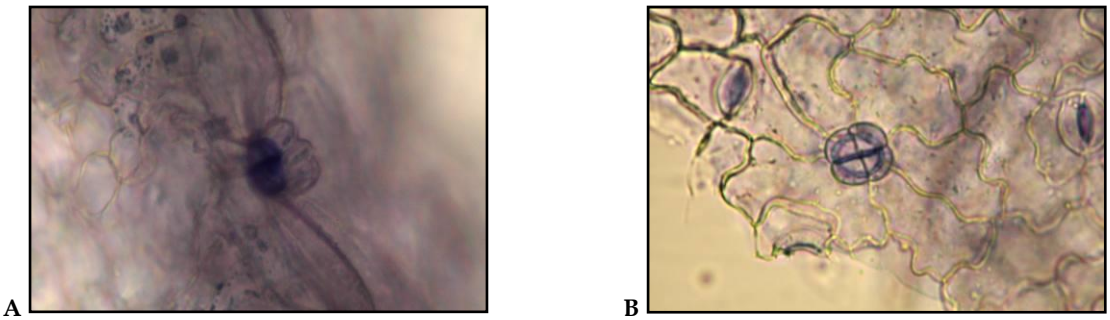

Fig. 4. Unbranched glandular hair from leaf: A-cross-section (ob. 40x); B- surface preparation (ob. 40x)

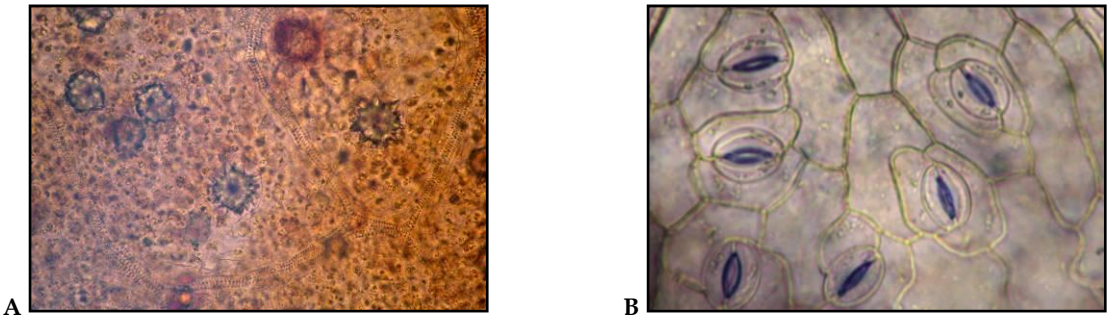

Fig. 5. Leaf - surface preparation: A- calcium oxalate druses (ob. 10x); B- paracytic type stomata (ob. 40x)

Coss-sections of leaf revealed four co-llateral closed vascular bundles enclo-sed in sclerified pericycle in the main rib (Fig. 3 A) and heterogeneous asy-mmetric leaf blade structure with sec-retory cavities (Fig. 3, B) and unbranched four cells glandular hairs (Fig. 4 , A). In the leaf surface preparation were observed glandular hairs (Fig. 4, B), calcium oxalate druses (Fig. 5, A) and paracytic type stomata (Fig. 5, B).

Due to abnormal distribution of root elongation values, ANOVA test could not be applied; the results were statistically processed using the Kruskal-Wallis test and the Dunn's post-test. Kruskal-Wallis test was applied to groups of obtained values (control vs. results for each dilution in 5 days).

For all test groups Kruskal-Wallis test showed statistically significant differences ($p < 0.0001$). For subgroup analysis we applied Dunn's test; this test allowed us to evaluate statistical signifiance of each subgroup (control vs. results for each dilution/each day); the results of statistical analysis are shown in table I.

The inhibitory effect compared to the control sample (Efi) was calculated using the formula [7]:

$$\mathbf{Ef_i} = 100 - \frac{P-1}{M-1}100$$

P – sample average of root elongation (cm)

M – control sample average of root elongation (cm)

1 – initial value of embryonic roots (cm)

100 – the results are expressed as a percentage

Efi values are also listed in table I.

Table 1. The inhibitory effect of analyzed solutions (PA and PE) compared to the control and statistical signifiance.

Sample	Ef_i (%)				
	24 h	**48 h**	**72h**	**96 h**	**120 h**
PA 5	97,71	98,96	99,26	99,44	99,51
Dunn's test	ns	ns	*	**	***
Kruskal-Wallis test			***		
PA 3,33	97,26	98,75	98,97	99,21	99,32
Dunn's test	ns	ns	*	**	***
Kruskal-Wallis test			***		
PA 2,50	96,80	98,33	98,68	98,99	99,13
Dunn's test	ns	ns	*	**	**
Kruskal-Wallis test			***		
PA 1,66	96,80	98,54	98,68	98,99	97,10
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		
PA 0,33	70,77	67,98	64,27	57,36	50,96
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		
PA 0,033	-9,58	7,90	8,19	2,90	2,60
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		
PE 5	99,08	99,58	99,56	99,66	99,71
Dunn's test	ns	ns	**	***	***
Kruskal-Wallis test			***		
PE 3,33	98,63	99,16	99,41	99,55	99,61
Dunn's test	ns	*	**	***	***
Kruskal-Wallis test			***		
PE 2,50	99,08	99,58	99,70	99,66	99,71
Dunn's test	ns	ns	**	***	***
Kruskal-Wallis test			***		
PE 1,66	98,63	99,16	99,26	99,55	99,61
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		
PE 0,33	92,69	90,02	88,43	86,49	83,68
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		
PE 0,033	36,07	16,42	26,79	24,10	25,86
Dunn's test	ns	ns	ns	ns	ns
Kruskal-Wallis test			***		

Legend
ns –not significant
***, **, *** - significant**

Symbol	**p value**
ns	> 0,05
*	0,01-0,05
**	0,001-0,01
***	< 0,001

Table 2. Observations made on karyokinetic film changes after 24h under the influence of analyzed solutions (PA and PE) at 5%-0,033% concentrations

Sample	**Observations**
Control (M)	- frequent divisions - all phases of mitotic division without changes
PA 5	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PA 3,33	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PA 2,50	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PA 1,66	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PA 0,33	- rare divisions - normal telophases, metaphases and anaphases - nuclei containing 2-3 hypertrophied nucleoli
PA 0,033	- frequent divisions - all phases of mitotic division without changes
PE 5,00	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PE 3,33	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PE 2,50	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PE 1,66	- absence of divisions, gel aspect cellular content - disorganized nuclear material - abnormal shaped nuclei
PE 0,33	- rare divisions - telophases and metaphases in tropokinesis, bridge anaphases, anaphases with retarded chromosomes - nuclei containing 2-3 hypertrophied nucleoli
PE 0,033	- frequent divisions - all phases of mitotic division without changes

Changes in karyokinetic film examined by microscope after 24 hours are listed in table II and they can also be observed in fig. 6, A-D.

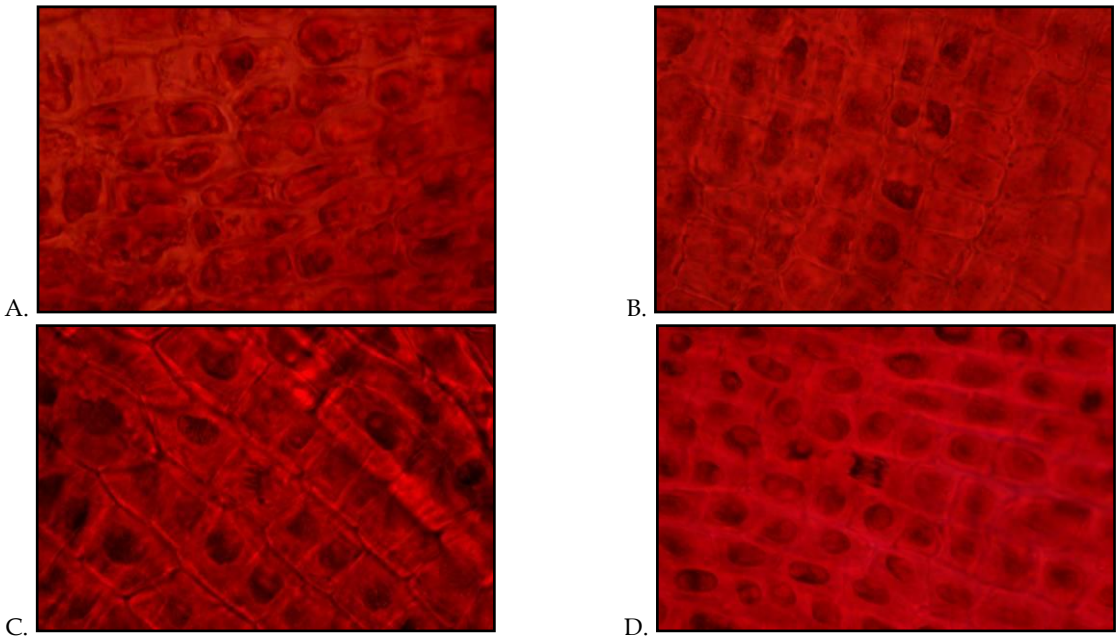

Fig. 6. Karyokinetic film changes induced by PA and PE: A- gel aspect cellular content, disorganized nuclear material, abnormal shaped nuclei; B- disorganized nuclear material, abnormal shaped nuclei; C- metaphase in tropokinesis; D-bridge anaphase (ob. 40x).

DISCUSSIONS

Microscopic examination revealed: cortical parenchyma with air channels, sclerified pericycle in the stem and calcium oxalate druses, secretory cavities, unbranched four cells glandular hairs and paracytic type stomata.

Triticum test showed a strong inhibitory effect of both solutions analyzed at concentrations 5%, 3.33%, 2.5%, 1.66%: between 96.8% and 99.5% for the aqueous solutions and between 99.1% - 99.7% for the ethanolic ones. These results are statistically significant and highlighted by the cytotoxicity observed in microscopic preparations. At 0.33% concentration, both extracts inhibit almost constantly, but not statistically significant: aqueous solution ratio of only 50.9% - 70.7% and ethanolic solution between 83.7% and 92.7%. Although the results obtained at 0.33% concentration is not statistically verified, the biologically effect may be important [7]. Given the microscopic observations of karyokinetic film changes (disorganized nuclear material, abnormal shaped nuclei, metaphases in tropokinesis, bridge anaphases) we can conclude that both solutions at 0.33% concentration inhibit mitotic division. At the concentration of 0.033% the result was an inhibition of only -9.6% - 8.2% for the aqueous solution and 16.4% - 36.1% for the ethanolic solution.

CONCLUSIONS

Polygonum hydropiper L. has cytotoxic compounds soluble in both water and ethanol. Therefore we consider necessary to further research, both to determine the toxicity of it's extracts on genetic material in the event of pro-

longed administration of infusions or other extracts, and because of it's therapeutic potential in anti-tumor therapy.

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