

RESEARCH REGARDING THE THERAPEUTIC VALORISATION OF THE PRODUCT *ALLII CEPAE* *BULBUS* - CYTOTOXICITY EVALUATION

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Abstract

The literature researches on the species *Allium cepa* L that placed them among the products with antimitotic properties, determined us to test the cytotoxic action of several extractive fractions (estimated by the phytobiological method Constantinescu - *Triticum* test) for detecting the pharmacologically active fraction.

Were assessed the hydrodistilled extract (CH), aqueous macerate (CM) and fresh juice (CS) in different concentrations, compared with colchicine (RC) and methotrexate (RM) (control sample (M) = distilled water). There were investigated: the influence on wheat caryopsis germination, root elongation and changes in mitotic film (after 24 hours – with the Nikon 2 Labophot microscope).

We observed the inhibition of germination for CM, CS, RC, RM, and its stimulation in the case of CH, the inhibition of root elongation by CM, CS, RC, RM, or its stimulation by CH, and changes in the mitotic film (nucleus and nucleolus hypertrophy, placed into the cells corners, precipitated nuclear material). All these results demonstrated the cytotoxic / cytostatic action.

In conclusion, only the aqueous macerate and the fresh juice of *Allium cepa* have cytotoxic properties.

Rezumat

Menționarea speciei *Allium cepa* L. printre produsele cu proprietăți antimitotice, ne-a determinat să testăm acțiunea citotoxică a mai multor fracțiuni extractive (prin metoda fitobiologică Constantinescu - testul *Triticum*), în scopul depistării celei farmacologic active.

S-au studiat: hidrodistilatul (CH), maceratul apos (CM) și sucul proaspăt (CS), în diferite concentrații, comparativ cu colchicina (RC) și methotrexatul (RM); martor (M) = apa distilată. S-au cercetat: influența asupra germinării cariopselor de grâu, elongația radiculară și modificările filmului mitotic (după 24 ore - la microscopul Labophot 2 Nikon).

În urma studiilor efectuate s-a observat inhibarea germinației (CM, CS, RC, RM), stimularea germinației (CH); inhibarea alungirii radiculare (CM, CS, RC, RM), stimularea alungirii radiculare (CH); modificări ale filmului mitotic (nuclei și nucleoli hipertrofiați, dispuși în colțurile celulelor, material nuclear precipitat). Acestea confirmă acțiunea citotoxică / citostatică.

Se poate concluziona că numai maceratul apos și sucul proaspăt din specia *Allium cepa* au efecte citotoxice.

Keywords: *Alli cepae bulbus*, mitoinhibitory action, inhibition index

Introduction

Psoriatic erythematous squamous lesions formed in delimited plaques, covered by thick scales, as a result of rapid division and incomplete maturation of dermal cells, and the use of antimitotic (methotrexate) and, newer, immunosuppressive agents (etanercept) in psoriasis treatment, led us to search for new vegetable sources of antimitotic substances with fewer side effects than methotrexate and etanercept used for this purpose [3,4,6,8].

The choice of onion bulbs - *Alli cepae bulbus*, is based on their antimitotic properties mentioned by D'Amato and Avanzi [1], subsequently confirmed by other authors [11].

As the cytotoxic/cytostatic constituents are not mentioned in the literature, in order to obtain pharmacologically active extracts, we considered necessary to test the extractive fractions for determining the most active.

Materials and Methods

As raw material there were used the bulbs of species *Allium cepa* L, onion (*Alliaceae* family), cultivated under bio-conditions, harvested at full maturity in August 2009, in Darvari-village, Ilfov County (Romania) and preserved at 10-15 °C.

For the determination of the possible cytotoxic or cytostatic properties it was used the D. Gr. Constantinescu method (*Triticum* test), by means of which we researched the influence of some extracts on wheat caryopsis germination, root elongation and changes in the mitotic film. The caryopsis used in the experiment were *Triticum aestivum* Mill., the variety Bohemia (Fam. *Poaceae*), purchased from SC Adaflor SRL.

Obtaining the solutions to be analyzed

Aiming to find the most active fraction, we prepared:

- a hydrodistillate - through steam distillation, concentration 5% (CH);
- a 5% aqueous macerate (CM);
- fresh juice obtained by pressing the fresh vegetal product (CS).

From these solutions, by dilution with water, different concentrations were obtained, noted by an index 1 to 5 (in order of decreasing concentration): for CM1 - CM5 and CH1 - CH5 (5, 3.33, 2.50, 1, 0.66 and 0.33 g vegetable product %), for CS1 - CS5 (100, 66.66, 50, 33.33, 6.66 g%).

As reference substances there were used: colchicine = RC1 - RC5 (0.20, 0.133, 0.066, 0.033 and 0.01 g%) and methotrexate = RM1 - RM5 (0.01, 0.001, 0.00066, 0.005, 0.00033 g%). As control sample (M) it was used distilled water. Reference substances concentrations were chosen based on the minimum inhibitory concentration (MIC) of germination [7, 10].

Wheat caryopsis and solutions to be tested were brought in Petri dishes, according to the Constantinescu method [5], later adapted by other researchers [2, 9]. To observe the mitotic film, there were used the coloration with *chlorhydric orcein* and a microscope Labophot 2 Nikon, with 100x objective, by immersion in cedar oil.

We assessed the influence on caryopsis germination for 3 days, root elongation for 5 days and mitotic film changes occurred after 24 hours.

Results and Discussion

Influence on the germination (Table I)

The influence on the wheat caryopsis germination of the *Allium cepae bulbos* products is detailed in Table I.

As it can be observed, there is an inhibition mechanism over the germination process for the samples noted as follows: CM1 - CM4, CS1 - CS5, RM1 - RM5, RC1 - RC5. Regarding the samples noted as CH1 - CH5, our results showed a stimulating effect over the wheat caryopsis germination, while the CM5 sample only developed an inhibiting effect during the first day of the experiment. According to the literature, this may be due to the instability of the sulfur compounds in a hydrodistillate.

Influence on the root elongation (Table II)

There is a total inhibition on the root elongation for the samples noted as CM1 - CM4, CS1 - CS5, RC1 - RC5, RM1 - RM5, throughout the experiment. In the same conditions, under the action of RC1 - RC5 there is little growth in the first two days, followed by stagnation of growth and thickening radicle (polyploidy phenomenon).

The *inhibition index* on the root elongation was calculated as:

$$I[\%] = \frac{L_m - L_t}{L_m} \cdot 100$$

where: L_m is the average root elongation of the control sample and L_t is the average root elongation of the treated sample.

Table I

The influence of the studied fractions on the germination, depending on their concentration

No.	Tested solution	Day 1 (cm)	Day 2 (cm)	Day 3 (cm)
1	CM1	0.27	0.28	0.33
	CM2	0.46	0.47	0.51
	CM3	0.61	0.61	0.73
	CM4	1.11	1.54	2.58
	CM5	1.33	2.55	4.60
2	CH1	1.21	2.51	4.64
	CH2	1.31	2.83	5.11
	CH3	1.38	3.10	5.31
	CH4	1.40	3.63	5.63
	CH5	1.42	3.85	5.95
3	CS1	0.20	0.23	0.27
	CS2	0.34	0.44	0.52
	CS3	0.43	0.51	0.60
	CS4	0.67	0.71	0.81
	CS5	0.80	1.03	1.16
4	RC1	0.33	0.37	0.44
	RC2	0.41	0.45	0.48
	RC3	0.49	0.53	0.56
	RC4	0.59	0.63	0.69
	RC5	0.64	0.72	0.79
5	RM1	0.44	0.58	0.60
	RM2	0.70	0.80	0.81
	RM3	0.83	0.87	0.92
	RM4	0.83	0.91	0.98
	RM5	0.84	0.96	0.99
6	M	0.98	2.53	4.94

Table II

The influence of the *Allium cepa* fractions on the root elongation, depending on the concentration

No.	Tested solution	Day 1 (cm)	Day 2 (cm)	Day 3 (cm)	Day 4 (cm)	Day 5 (cm)	Inhibition index [%]
1	CM1	1.04	1.09	1.12	1.17	1.23	81.10
	CM2	1.05	1.10	1.16	1.18	1.27	80.50
	CM3	1.06	1.11	1.17	1.19	1.28	80.34
	CM4	1.17	1.21	1.24	1.27	1.31	79.88
	CM5	2.69	3.70	4.79	5.47	6.39	1.84
2	CH1	3.19	4.90	6.21	7.23	8.29	-27.34
	CH2	3.25	4.92	6.25	7.25	8.31	-27.65
	CH3	3.28	4.95	6.37	7.28	8.35	-28.26
	CH4	3.34	4.99	6.49	7.67	8.71	-33.79
	CH5	3.41	5.56	6.81	7.87	8.99	-38.10
3	CS1	1.00	1.00	1.00	1.00	1.00	84.63
	CS2	1.00	1.01	1.02	1.03	1.04	84.02
	CS3	1.01	1.03	1.04	1.05	1.06	83.72
	CS4	1.02	1.04	1.06	1.09	1.13	82.64
	CS5	1.04	1.07	1.11	1.15	1.21	81.41
4	RC1	1.21	1.23	1.25	1.29	1.34	79.42
	RC2	1.44	1.49	1.51	1.54	1.58	75.73
	RC3	1.48	1.53	1.55	1.58	1.62	75.12
	RC4	1.59	1.64	1.67	1.69	1.70	73.89
	RC5	1.61	1.65	1.69	1.72	1.73	73.43
5	RM1	1.84	1.90	1.91	1.92	1.93	70.35
	RM2	1.94	1.95	1.96	1.97	1.98	69.59
	RM3	1.94	1.95	1.97	1.98	2.00	69.28
	RM4	1.95	1.96	1.98	2.00	2.02	68.97
	RM5	1.96	1.98	2.00	2.04	2.06	68.36
6	M	2.29	3.31	4.64	5.42	6.51	-

The influence on the cellular division is depicted in Fig. 1:

The hydrodistillate for the CH1 - CH4 sample dilutions stimulates cell division, and the dilution of CH5 has the same effect as the control sample (M).

The aqueous macerate for the CM1 - CM4 dilutions and for the fresh juice samples noted as CS1 - CS5 causes an inhibition of cellular division marked by disorganized metaphases (Fig. 1A), nucleus with hypertrophied large nucleolus, (Fig. 1B), telophase in tropocinesis (Fig. 1C).

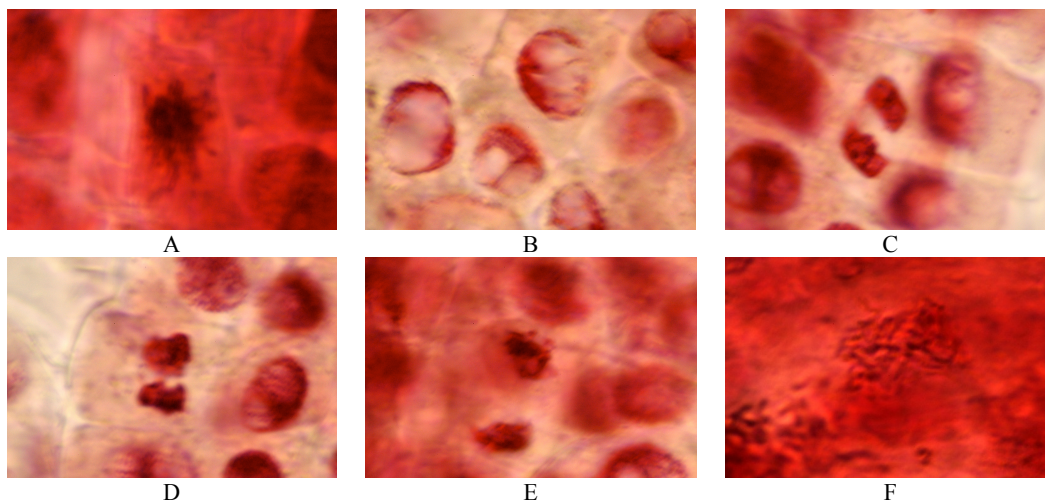


Figure 1

Mitotic film modifications under the influence of the aqueous macerate and fresh juice obtained from *Allii cepae bulbis*

Under the action of colchicine, at all concentrations it was observed a large number of chromosomal sets (polyploidy) with nucleolus of elongated shape, triangular and hypertrophied nucleus like dumbbells (Fig. 1D). They have a diffuse appearance of lobate nucleus (Fig. 1E).

Methotrexate effect resulted in the contraction of the nuclear material, nucleus with deformed nucleolus, hypertrophied and scattered chromosomes (Fig. 1F).

Mitotic film modifications (elongated and hypertrophied nucleus and nucleolus, chromosomes scattered, slightly contracted nuclear material) produced under the influence of test solutions CM1 - CM4 and CS1 - CS5, show a cytotoxic action, possibly cytostatic, of these extracts. Saponosides, polyholosides and flavonoids, chemically stable compounds, present in both

fresh juice and the aqueous macerate, could be the responsible for this effect.

The supposed cytostatic action of sulfur compounds is difficult to prove as long as they change their structure, soon after crushing the tissues, in the presence of aliinase by enzymatic hydrolysis (not chemically stable).

Conclusions

In conclusion, only the fresh juice and the aqueous macerate are cytotoxic (under their influence, no cell divisions occur and the mitotic film is altered), while the hydrodistillate does not influence cell division.

This is why we chose to use them as semi-finished products for obtaining pharmaceutical products with phytotherapeutic use (emulsions, creams), unfortunately with a short period of validity.

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