ANTIMITOTIC ACTIVITY OF SAPONINS OBTAINED FROM *ANAGALIS ARVENSIS*

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ABSTRACT. Because of the extended use of plant derived drugs (Tămaş, 1995), we studied some plant extracts and substances obtained from plants (Tămaş et al., 1978 et al., Tămaş et al., 1983). Natural drugs are used on an extended basis because of limited secondary effects and adverse reactions. In our present paper we tested the action of saponins obtained from *Anagalis arvensis* (Boiteanu et all, 1964, Glombitza and Kurth, 1987, Amoros and Girre, 1987) on the growth of meristems of *Triticum sp.* and cellular division. Tests with saponins obtained from *Anagalis arvensis* inhibit the growth of *Triticum sp.* meristems, and cellular division, concluding that these saponins may be included in the antimitotic medication group (Schonfelder and Schonfelder, 2004).

Keywords: saponins, *anagalis arvensis*, antimitotics

INTRODUCTION

The vegetal test may bring valuable information referring to the cytostatic, and mitotic inhibition activity of some plant extracts and the bioactive substances isolated from them. Because plant cells represent a more confortable test material, due to the lower degree of differentiation than animal cells, many cytostatics have an inhibitory mitotic effect on them, rather than a cromatoclasic effect observed at higher concentrations.

The inhibition coefficient on the growth of meristems of *Triticum* sp. offer precious information about cytostatic substances.

In order to proceed with screening of a cytostatic substance this has to have an inhibition coefficient of over 50%. Phytobiologic tests are very sensitive and the results obtained offer informations about the mechanism of action of certain substances on metabolic processes taking place in cells or tissues.

Data obtained about the influence of saponins on the growth of meristems of *Triticum* sp. revealed a strong inhibitoy growth effect, which may lead us to the conclusion that saponins are inhibiting cellular division. That is why we examened by microscopy, cells from the roots of *Vicia faba* kept for 24 hours in saponin solutions of *Anagalis arvensis* with concentrations of 0,1 and 0,01%.

MATERIALS AND METHODS

We determined the inhibiton coefficient (Ariesan et al, 1084, Tămaş, 1976) on meristems of *Triticum* sp. treated with saponins from *Anagalis arvensis* compared with the Merck saponin.

The biological material is represented by caryopses of - *Triticum vulgare Will* (*Triticum aestivum L.*), which were set for germination in Petri plates at temperatures around 25 ^{0}C. The solutions we analized consisted of saponins of *Anagalis arvensis* analized by comparing it to a solution 0,02% of saponin Merck. 24 and 48 hours after the treatment of the seeds we read the length of the rootlets and calculated the inhibition coefficient.

In order to test the activity of the saponin obtained from *Anagalis arvensis* on cellular division we used the histological technique (Serbanescu et al., 1983, Tarnavschi et al., 1974, Tarnavschi et al, 1981). This technique consists in keeping the rootles from the freeshly germinated seeds in Petri plates with solutions of saponins for 24 hours. In parralell with this we prepared a blank probe conisting of freshly germinated seeds with the rootlets kept in water. After 24 hours the probes are treated with a mixture of alcohol and acetic acid (3/1), and kept for 24 hours in the refrigerator, after which these are prepared by a histological technique, colored with hematoxilin-eosine or R. Brachet and examined by microscopy observing the aspect of the nucleus during the interphase and the cytoplasm.

RESULTS AND DISCUSSION

24 hours and 48 hours after the treatment of seeds we measured the length of the roots in mm and calculated the inhibition coefficient for each of the group we treated. The results are presented in the table bellow:

Table 1

AVERAGE ROOTLET LENGTH AND INHIBITION COEFFICIENT

Probe	Concentration	Average rootlet length (mm)		Inhibition coefficient (I)	
		24h	48h	24h	48h
blank		11,4	31,4		
Saponin *A. arvensis*	0,01%	5,2	7,2	54,38%	77,07%
Saponin Merck	0,02%	8,5	17,2	25,43%	26,22%

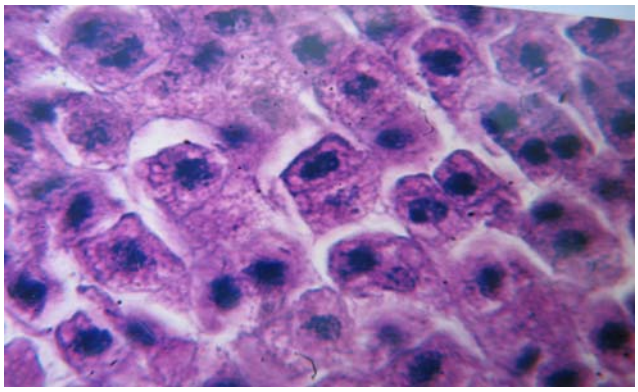

Fig. 1 Blank probe

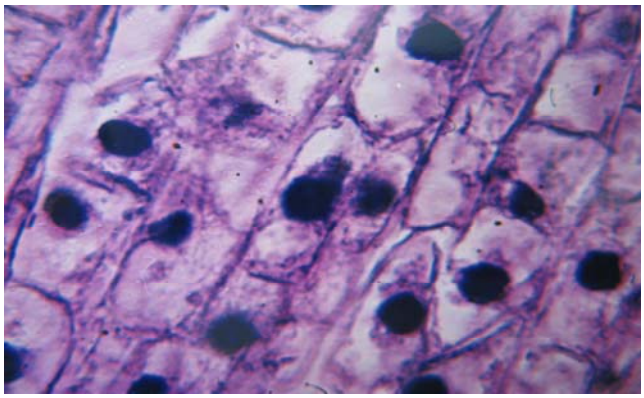

Fig. 2 Probe treated with saponin from *A.arvensis* 0,01%

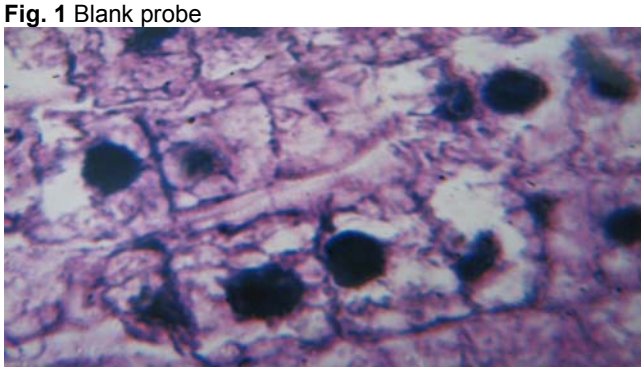

Fig. 3 Probe treated with saponin from *A. arvensis* 0,1%

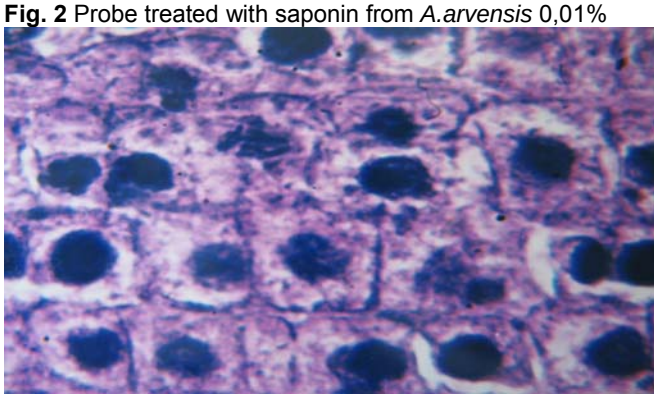

Fig. 4 Probe treated with saponin from *A. arvensis* 0,1%

Probes prepared by the histologic method are examed by microscopy observing cells in the process of cell division compared with a blank probe.

Figure 1 presents the normal aspect of a longitudinal section of the meristeme, wihth the nuclei in interphase or other phases of cellular division, with dense cytoplasm, colored in light red, and the nucleus colored in dark purple.

The probe treated with saponin 0,01% (figure 2) presented cytoplasmic vacuolizations (white vesicles), uneven diformed nuclei and contracted chromatin, very dense. Other changes can also be seen and consist of changes of the cell wall which takes the aspect of a „zig-zag" line, but essential is the absence of the division fragments.

At concentrations higher than (0,1%) more profound changes are noted, with lighter colour around the nucleus, a white halo, the nuclei are condensed in chromatine, appear very dark and fragments of divisions are lacking. (figures 3 and 4).

We can explain this by the fact that saponins interact with cell membranes, mainly whith the tonoplast, producing unevenly distributed vacuoles wihin it. Saponins also act on the nucleus membrane destroing its structure and function of selective and specific transport between the cytoplasm and nucleus, resulting in the arrest of cell division and malfunctions in many cell compartiments.

CONCLUSIONS

Readings of growth of meristems were performed at 24 and 48 hoous after treating the seeds of *Triticum sp*. with the analized substance. The inhibition coefficient for the meristems treated with saponins obtained from *A. arvensis* is 54,38 % after 24 hours, and 77,07% after 48 hours from the contact with the saponin solution.

We observed that the inhibition coefficient after 24 and 48 hours was higher for meristems treated with saponins from *A.arvensis* compared with those treated with the Merck saponin.

Concerning the activity of saponins from *Anagalis arvensis* on cellular division we noted the inhibitory effect because of the interaction with the membrane lipids thus blocking the normal metabolic processes including cellular division.

Thus, these saponins can be included in the potential antimitotic group, in the class of mitosis inhibitors, fact which explains why their activity intensity depends on their concentration.

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