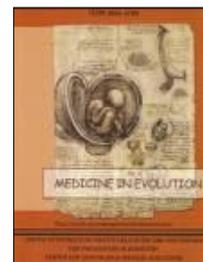


CONTRIBUTIONS TO THE STUDY OF SOME SPECIES IN THE PORTULACA (PORTULACACEAE) GENUS. PRELIMINARY BOTANICAL AND PHYTOBIOLOGICAL RESEARCH ON PORTULACA OLERACEAE L AND PORTULACA GRANDIFLORA HOOKER SPECIES



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ABSTRACT

Aim and objectives – The aim of this research was to verify the identity and the possible cytotoxicity of *Portulaca oleracea* and *P. grandiflora* species. This study is the first step in use these species to development of pharmacologically active extracts.

Material and methods – The studied species were harvested from Bucharest (2007, September – 2010, October). The identity of the species was checked macroscopically and microscopically (with Labophot II Nikon microscope) and the cytotoxicity of the aqueous and ethanolic extractive solutions was verified using the Constantinescu phytobiological method. The results were statistically assessed using the Anova and Kruskal-Wallis test.

Results – The macroscopic and microscopic analysis confirmed the identity of the species.

The phytobiological testing revealed the significant inhibitory effect on the radicular growth at high concentrations for all the extracts. Changes of cellular division were observed.

Conclusion – The results of the phytobiological testing indicate the antitumor potential of the studied species.

Key words: *Portulaca* sp, phytobiologic, cytotoxic, botanical

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INTRODUCTION

The *Portulaca* genus includes about 100-125 species. Among these we have focused our attention on *Portulaca oleracea* L. and on *P. grandiflora* Hooker. *Portulaca oleracea* L. (pursley) is a resistant herbaceous plant which grows spontaneously in all the regions of Romania (Bucharest, Oltenia, Muntenia, Dobrogea) and it is commonly used as a food source. It is frequently met in areas with hot or temperate climate. It is a wild species, but also an aggressive weed, having a higher content of antioxidant compounds and omega-3 fatty acids compared to other vegetal species that were studied so far¹.

Because last characteristic *P. oleracea* appreciate mostly for its nutritive value rather than for its invasive action (as a weed).

For a long time, it has been and it is still used as food in some regions². At the moment it is used in the feeding of the poultry to reduce the cholesterol from eggs. The leaves are used to flavour some cheese and mayonnaise types. The plant is used in the traditional medicine for the anthelmintic, antitumor, antimicrobial and diuretic actions. *P. grandiflora* Hooker is known as a decorative plant. The literature presents researches that confirm the antimutagenic, in vitro lymphoma stimulating and analgesic properties⁴ of *P. grandiflora* species. Among the numerous pieces of information on the species of the "Portulaca" genus in Medline database very few refer to the botanical characteristics and none of them discusses the influence of different extracts on cellular division.

AIM AND OBJECTIVES

The objectives of our research are to verify the identity and the possible cytotoxicity of the selected species,

with the purpose of using them in the preparation of some pharmacologically active extracts.

MATERIAL AND METHOD

The raw material that was used, represented by the *Portulaca oleracea* and *Portulaca grandiflora* plants, was harvested from Bucharest (Al. I. Cuza Park and the Botanical Garden of "Carol Davila" University of Medicine and Pharmacy) between 2007, September and 2010, October. One specimen harvested from each species has been kept in the dessicated collection of the Pharmaceutical Botany laboratory of the Faculty of Pharmacy, "Carol Davila" UMP, Bucharest.

The identity of the vegetal products was verified through macroscopic and microscopic examinations (on

transverse sections through the root, the strain and the leaves, which were double-stained and on surface preparations from leaves, fruits and seeds, which were clarified with sodium hydroxide solution 50g/L). The Labophot II Nikon microscope was used (10x eyeglass and 10x, 40x object glasses). The morphological and anatomical characteristics that were observed were compared to data from the literature.

The phytobiological testing (the Constantinescu method - the Triticum test) was based on the determination of the maximum dilution of the extractive solution which, depending on the pe-

riod of action, influences the radicular growth and the nucleokinetic film ⁵. Embryonic roots from wheat *Triticum vulgare* Mill, *Drobia* were used. The caryopses of wheat were obtained from Fundulea Agricultural Research Institute. Aqueous and alcoholic extractive solutions from the aerial part of *Portulaca oleracea* L and *Portulaca grandiflora* Hooker were prepared for the testing. Solutions for analysis of 5g%, 2.5g%, 1.66g%, 0.33g%, 0.033g% concentrations were obtained through dilution from the extractive solutions. These were added in Petri dishes (15 cm diameter) containing caryopses that had germinated in laboratory conditions, having 1cm long embryonic roots.

The germinated caryopses have stayed in contact with the analyzed

solutions for 5 days and the radicular growth was measured during this period.

The control sample was prepared in the same way using only distilled water. For the microscopic exam the embryonic roots from one caryopsis in each Petri dish were sectioned 5mm far from their top (after a contact of 24 hours with the analyzed solution).

The sections were coloured with diluted acetic orcein (because the orcein has high affinity for chromatin in acidic medium) and were examined using the Labophot II Nikon microscope with 10x eyeglass and 100x objective glass with cedar oil immersion.

The statistical assessment of the results was conducted using the Anova and Kruskal-Wallis test ($p < 0.0001$).

RESULTS

The macroscopic analysis has confirmed the morphological characteristics of *Portulaca oleracea* L and *Portulaca grandiflora* Hooker species, described in the Romanian Flora ⁶.

The microscopic analysis of the transverse sections through the root has outlined a secondary structure at both species (fig.1). The root from *P. oleracea* presents multiseriate medullary rays in the secondary phloem and xylem (fig.1B).

The strains from both species have a primary structure, but depending on the development stage an incomplete secondary structure could be observed on some sections from both species (fig. 2C). The conducting bundles of *P. Oleracea* form groups of 2-3 (fig.2A) and the ones of *P. grandiflora* are individual (fig.2B).

The leaves have Krantz anatomical structures ^{7, 8} that are differently shaped. *P. oleracea* has presents collateral zigzag conducting bundles, while in

the case *P. grandiflora* the bundles follow the shape of the section which is elliptical and also there is a bundle in the centre of the section (fig.3B). The tector hairs are big, multicellular and they appear only for *P. grandiflora*. The stomata are paracytic for both species (fig.3C).

All the organs of both species contain calcium oxalate druses (fig.4C).

The surface preparations from the flower have outlined the presence of pollen grains having echinulate exine for both species (fig. 4A and B).

The phytobiological testing has revealed an inhibitory effect on the radicular growth at 5%, 2.5% and 1.66% concentrations which appears in all the extracts after 48 hours. The aqueous extracts are more potent than the alcoholic ones. The inhibitory effect is more powerful for *P. oleracea* than for *P. grandiflora*. The inhibitory effect of the 1.66% alcoholic extract of *P. oleracea* is not statistically significant. A stimu-

ting effect can be also observed, depending on the concentration (0.33%, 0.033%), but this effect is statistically significant only for the 0.003% aqueous extract and for the 0.033% alcoholic extract of *P. oleracea* (fig 6).

The changes of the cellular division are represented by: nuclei with hypertrophied nucleoli, anaphases with retarded chromosomes, metaphases and telophases in tropokinesis (fig.5A and B).

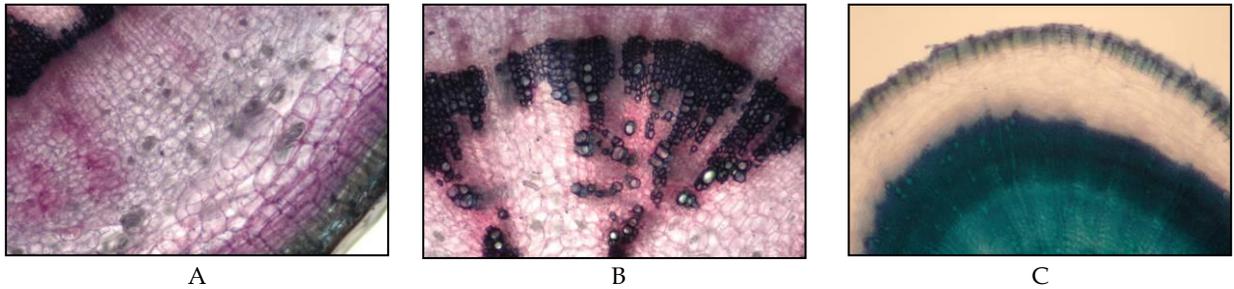


Fig. 1. Root structure from *P. oleracea* (A și B) and from *P. grandiflora* (B și C) (ob.10x)

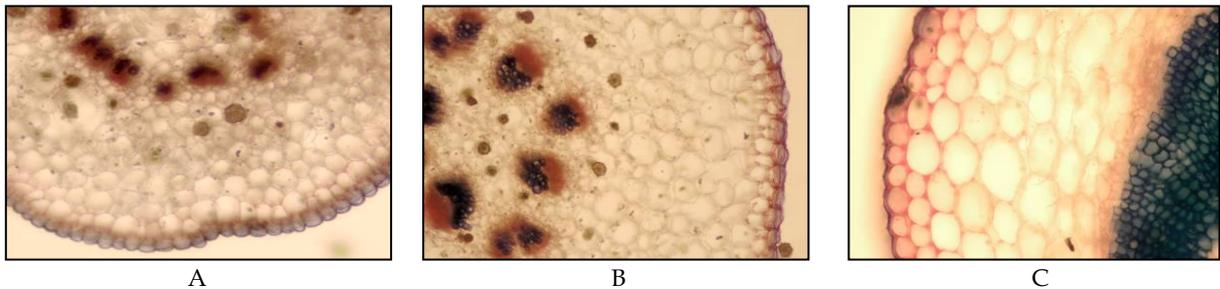


Fig. 2. Strain primary structure from *P. oleracea* (A) and from *P. grandiflora* (B); strain secondary structure *P. grandiflora* (C) (ob.10x)

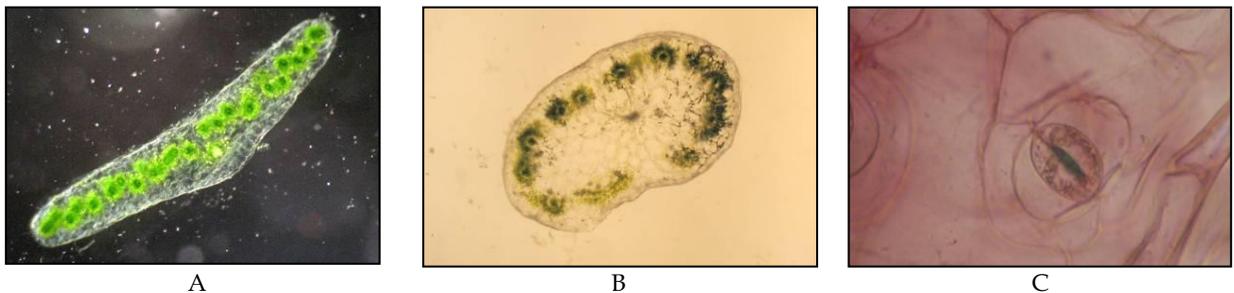


Fig. 3 Leaf structure from *P. oleracea* (A) and from *P. grandiflora* (B) (ob.4x); Paracytic stoma (ob.40x) (C)

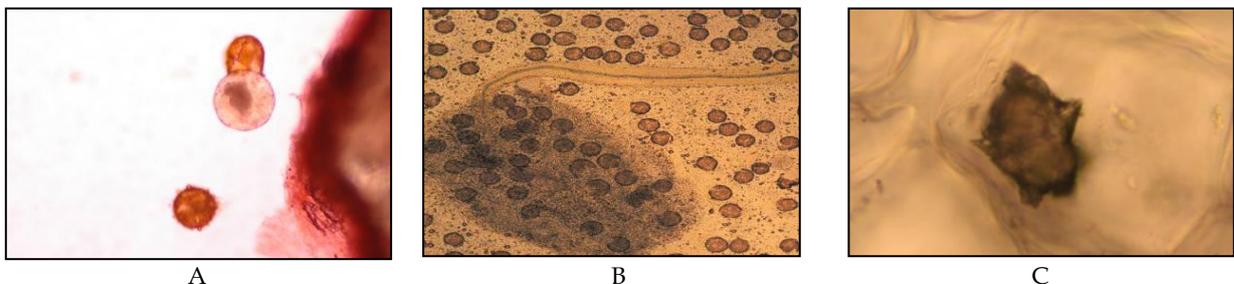


Fig.4 Pollen grains from *P. oleracea* (A) (ob.40x) and from *P. grandiflora* (B) (ob.4x); druse (C) (ob.40x)

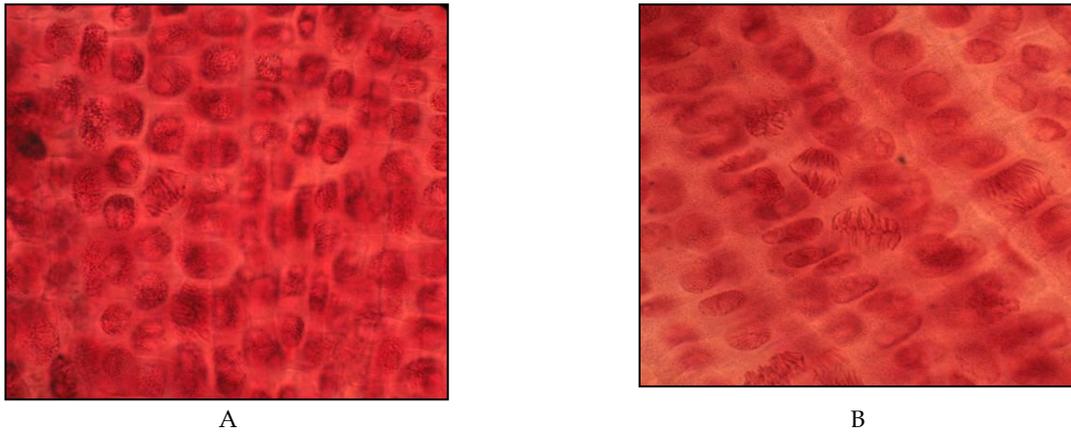


Fig. 5 Changes of cellular division: nuclei with hypertrophied nucleoli and anaphase bridges (A), anaphase with retarded chromosomes, tropokinetic metaphase (B) (ob.40x)

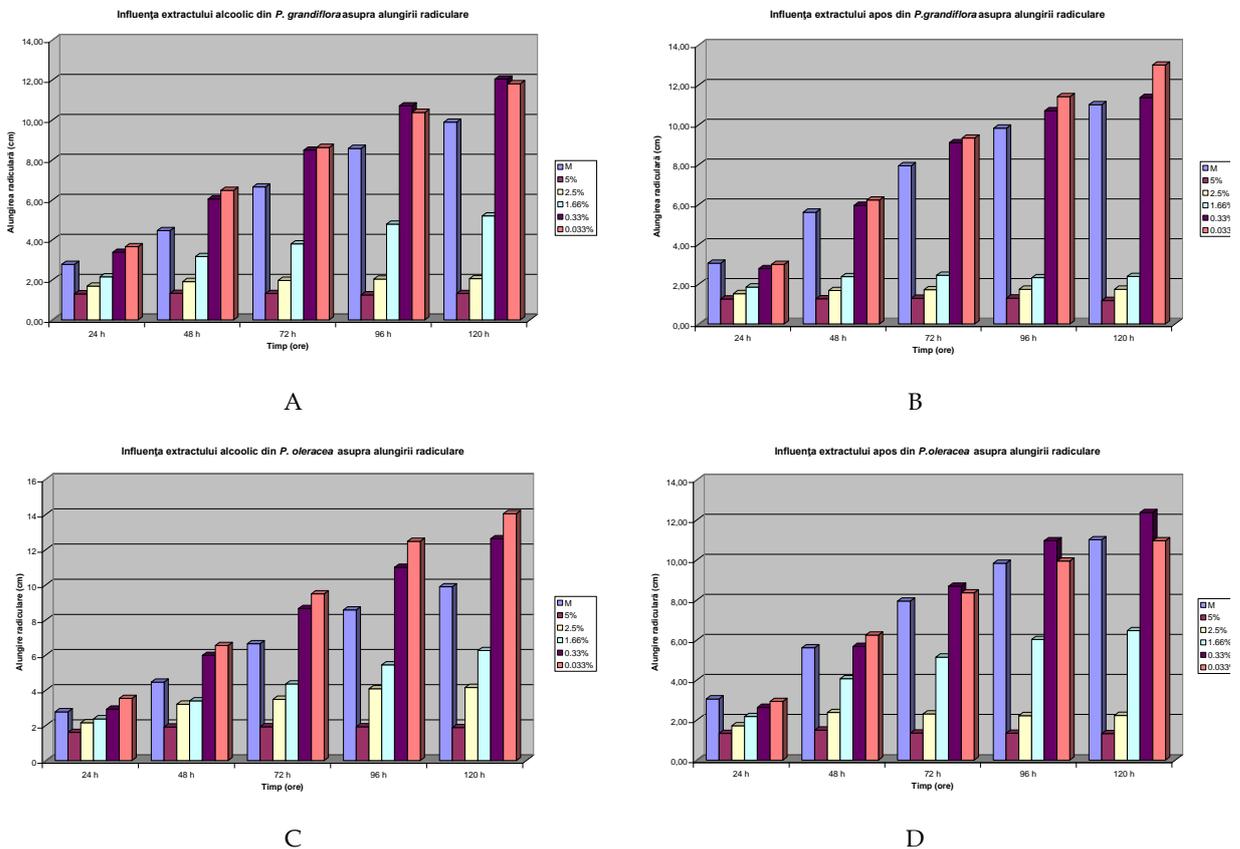


Fig. 6 Influence on root elongation of the extracts: alcoholic (A), aqueous (B) of *P. grandiflora* and alcoholic (C), aqueous (D) of *P. oleracea*

DISCUSSIONS

The identity of the two species (*P. oleracea* and *P. grandiflora*) can be microscopically and macroscopically de-

termined. Although they present many morphological differences, they are less different anatomically. The results of

the phybiological testing suggest that the analyzed solutions have a cytotoxic effect, which can justify its traditional using as an antitumor agent.

Because of these changes we find it necessary to raise a warning on a moderate use of *P. oleracea* in the nourishment of people and animals.

CONCLUSIONS

Since the consulted literature does not present data referring to the microscopic structure of the roots of these species, we consider this research to be a modest contribution to the knowledge of the anatomic characteristics of these species. We also consider our suggestion of a possible antitumor poten-

tial as another modest contribution to the knowledge of *P. grandiflora* species. To further investigate the antitumoral effect of *P. grandiflora* more research using normal and tumor animal cells cultures (off different types) as well as experimental animals with graft tumors is needed.

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