DETERMINING THE AMOUNT OF NUCLEIC ACIDS IN MEDICINAL FERNS COLLECTED FROM DIFFERENT AREAS OF ARAD AND BIHOR COUNTY

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Abstract

Lycopodium clavatum L. and Equisetum arvense L. are ferns, Pteridophytes, and are widely used medicinal plants. These species are not cultivated in Romania, the medicinal product coming from traditional collection centers, so the exact origin of the product is difficult to follow. In this work we are presenting the obtained results of determining the amount of nucleic acids in case of several Lycopodium clavatum and Equisetum arvense L. populations, in terms of the species' homogeneity.
There were studied the stems of Lycopodium clavatum L. and Equisetum arvense L., collected from different regions - Oradea's area, Cefa area - of Bihor County, and from Macea Botanical Garden (MBG), Arad county in the period of April - September 2014.
As a result of the study we have observed the presence of differences in the amount of nucleic acids between the studied populations.

Keywords: Lycopodium clavatum L., Equisetum arvense L., nucleic acids

INTRODUCTION

Pteridophytes are known from as far back as the Silurian, or some 380 million years ago (Bennici, 2008, Gifford et al, 1988). The Pteridophytes occupy the intermediate position between the bryophytes and the phanerogams (Graham, 1993).

The Pteridophytes origin theories fall into two general categories, those which postulate a direct origin from algae and those which consider pferidophytes directly or indirectly related to bryophytes.

Although their origin is much discussed, today this phylum is represented by a small number of species (Raven et al, 2005, Pooja, 2004, Pryer et al, 2004, Bailey et al, 1976).

Lycopodium clavatum L. and *Equisetum arvense L.* are Pteridophytes. They are widely used medicinal species. Both species are considered living fossils, which in many countries are protected plants (Smith et al, 2006, Raven et al, 2005, Rollinger et al, 2005).

Lycopodium clavatum L.: Lycopodiaceae family, Lycopodiales order Lycopodiopsida class.

It is an evergreen plant of wild flora, which grows in Europe and North America, in some countries being protected by law. In Romania it is

found at altitudes above 600 meters at the edge of forests or coniferous forests. They do not stand direct sunlight, they prefer shade, cool and moist soils.

It is a perennial herb with creeping stems up to 1 m long and with a height of 15-20 cm. It presents adventitious roots, dichotomically branched . The bifurcated branches bearing the top two spore cones and it rises from creeping stems. The small and dense leaves have a linear form, are arranged spiral on the branches. Each ear is wearing sporangium with stretched base covering each other. The sporophyton show, on the top, one large sporangium, kidney-shaped, in which tetrahedral spores was formed (Hoffman et al, 2002, Toma, 1998, Tamas, 1990).

In phytotherapeutic purpose they use the entire aerial part of the plant and spores that must be harvested in July-August, before you shake. Because a plant reaches maturity hard, it must be taken care not to elicit with the root and not to be broken the creeping stems (Bone et al, 2012, Petri, 1979).

Lycopodii sporae is used for manufacture of tablets, rarely for homeopathic tinctures.,

Lycopodii herba contains alkaloids, triterpenes, flavonoids. It is indicated for the treatment of rheumatoid arthritis, urinary disorders, liver disease, to combat alcohol and nicotine dependence (Hoffman et al, 2002, Tamas, 1990).

Equisetum arvense L.: Equisetaceae family, Equisetales order, Equisetopsida class.

Equisetum arvense L., has extensive and deep-seated rhizomes that are dark brown to black, dull, covered with hairs and occasionally bear tubers. The tubers are formed as shortened swollen internodes consisting of starch-filled cells traversed by a few vascular bundles. The rhizomes send out shoots each year. The sterile stem is circular, with 6-18 ribs and as many of valecules (Brune, 2008, Kozak, 2007, Borg et al, 1967, Bir, 1960).

Equiseti herba as constituent of diuretic teas; it has antiseptic action of urinary system, haemostatic, healing and mineralizing (Romanian Farmacopeia, 2008, Kozak, 2007).

MATERIALS AND METHODS

We were determining the amount of nucleic acids in case of several *Lycopodium clavatum L.* and *Equisetum arvense L.* populations, in terms of the species' homogeneity.

There were studied the stems of *Lycopodium clavatum L.* and *Equisetum arvense L.*, collected from different regions - Oradea's area, Cefa

area - of Bihor County, and from Macea Botanical Garden (MBG), Arad county in the period of April - September 2014.

The amount of nucleic acids was determined with Spirin Method.

For the quantitative determination of nucleic acids in various tissues the Spirin method may be used (Pallag et al, 2004, Spirin, 1959).

The nucleic acids are extracted from biological material by treating them with hot perchloric acid solution. The hydrolysis of nucleic acids with formation of soluble fragments is comlpete and their extraction from the tissue is of quantitative and is performed simultaneously.

In other methods such as the method Schmidt-Thannhausser and Webb, the quantitative determination of nucleic acids is carried out by hydrolysis of preparations obtained by prior processing of the tissue.

Alkaline or acidic hydrolysis are essential because the preparations of unhydrolyzed nucleic acids , at wavelengths corresponding to the ultraviolet region of the spectrum, have different optical density according to the state of aggregation of the mixture and the pH of the solution.

The preparation of nucleic acids from the analysed material, by treating it with trichloroacetic acid, in order to remove tissue components acido solubles are inconvenient, if the dosage thereof in the ultraviolet spectrophotometry, due to the trichloroacetic acid that has the maximum ultraviolet absorption in the region of 240-300 mμ.

Maximal absorbtion for DNA preparations is at 268 mμ and 260 mμ for RNA, namely in the same region that is characteristic for trichloroacetic acid.

The elimination of this drawback can be achieved when trichloroacetic acid is replaced in the procedure for the preparation of nucleic acid, perchloric acid.

The use of perchloric acid, allowed the improvement method of determination of nucleic acids.

Materials

- centrifuge (5000-6000 rev / min.).
- centrifuge tubes (20 ml.)
- bath water
- Spetrophotometre in ultraviolet
- tissue samples
- perchloric acid ($HClO_4$) 0.5 N

Procedure:

In several centrifuge tubes with a capacity of 20 m it is introduced 10.5 ml perchloric acid, 0,5 N and tissue samples whose weight ranges from 5 to 500 mg (based on the amount of nucleic acid in the examined tissues).

After careful shaking, the samples are placed in a boiling water bath (100 ° C), where they are kept for 20 minutes. Then the samples are cooled

under a stream of tap water centrifuged at 5000-6000 rev / min. for 10 minutes.

The supernatant is used for reading samples from spectrophotometer.

The total optical density of hydrolyzate containing both RNA and DNA is in function of the resulted components' concentration, after acid hydrolysis and the quantitative ratio of the two nucleic acids.

If the total amount of hydrolysed nucleic acid in the sample is high , beyond the optimum concentration for reading spectrophotometer, then the samples are diluted with 0.5 N perchloric acid solution.

Working tests are carried out at two different wavelengths 270 mμ and 290 mμ, using quartz cuvettes of 10 mm, hydrogen lamp and the witness sample 0.5N perchloric acid solutions.

The difference between the values read on the same sample at the two wavelengths and divided by the index of 0.19, indicates the phosphorus concentration of a nucleic ml. solution (in mg.).

Index 0.19 is the specific extinction is the same for both DNA and RNA and has been found experimentally by Spirin, who used standard solutions of the two acids, in which the nucleic phosphorus concentration of 1 mg phosphorus was a solution ml.to the thickness of 10 mm (Pallag et al, 2006, Pallag et al, 2004, Spirin, 1959,).

The formula used for calculation is

$$Cg\ (P\text{-}AN) = \frac{D\ 270 - D290}{0{,}19}$$

Cg(P-AN) - the concentration of nucleic phosphorus, expressed in g
0,19 - the index of extinction
D270 - the optic density of the hydrolyzate at 270 mμ
D290 - the density of hydrolizate of RNA and DNA at 290 mμ

RESULTS AND DISCUSSION

Plants harvested from different areas were noted:
Oradea area – Bihor County - **LO**
Cefa area – Bihor County - **LC**
Macea Botanical Garden (MBG) –Arad County - **LM**

Results can show in Figure 1 and 2.

Significant differences have been observed in the amount of nucleic acids at the plants studied about, from different populations which can also cause differences in the harvested and used medicinal product quality.

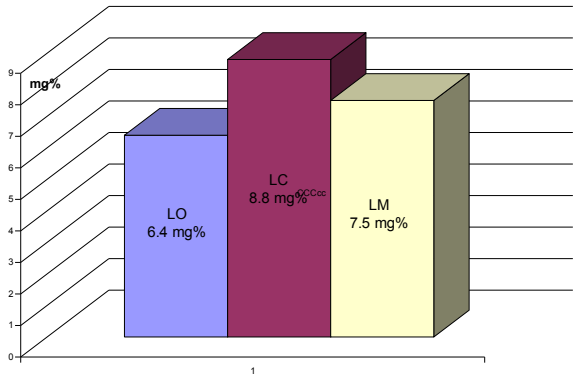

Fig.1. The amount of nucleic acids in Lycopodium clavatum L.

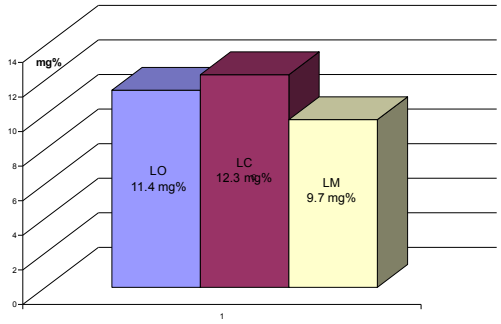

Fig.2. The amount of nucleic acids in Equisetum arvense L.

CONCLUSIONS

In this study two species of ferns have been included used in phytotherapy, but at the same time are representatives of some groups of plants, are very few species in the current flora.

As a result of analysis significant differences have been observed in the amount of nucleic acids at the plants studied about, from different populations which can also cause differences in the harvested and used medicinal product quality.

Introduction in culture of these species, should be taken in consideration, this will ensure the quantity and quality of medicinal product, used in phytotherapy as reqiured but the survival of these species can be ensured, these are considered living fossils in Romania's spontaneous flora.

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