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SPRING DRUGS OF *BETULA PENDULA* ROTH.: BIOLOGIC AND PHARMACOGNOSTIC EVALUATION

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ABSTRACT. The vegetal products harvested at spring time from the birch tree, known as spring elixirs, are the birch sap (*Betulae limfa* or succus) and the birch tree foliar buds (*Betulae gemmae*). This study was concerned in gathering information regarding the harvest process of the birch sap, organoleptic description of both the sap and foliar buds, as well as regarding diuretic and uricosuric activity (in vivo, on animal model) and the antiproliferative effect (phytobiologic test on *Lepidium sativum* grains). Diuretic and uricosuric activity of the birch sap is superior compared to the bud extracts and the antiproliferative activity evaluated through the inhibition of germination of Lepidium sativum grains, which can indicate a possible antitumoral activity of several dilutions of the birch sap and aqueous extract from birch foliar buds.

Keywords: birch, succus, buds, diuresis, *Lepidium sativum*

INTRODUCTION

Birch is the name of any tree of the genus, in the family *Betulaceae. Betula pendula* Roth. (Silver Birch) the most frequent species found in Romania is the widespread European birch, well known for its therapeutic properties. It is a medium-sized deciduous tree with a crown of arched branches with drooping branchlets. The bark is white, often with black diamond-shaped marks. The shoots are rough with small warts, and hairless, and the leaves 3-7 cm long, triangular with a broad base and pointed tip, and coarsely double-toothed serrated margins (Antal, 2009; Istudor, 2001).

The silver birch tree is the medicinal plant, from which during the whole biologic annual cycle providing the most numerous vegetal products used for therapeutic purpose: in spring time - sap- (*Betulae limfa* or *succus*), foliar buds (*Betulae gemmae* in gemmotherapy), in summer time - leaves (*Betulae folium*), external bark (*Betulae cortex*), and in autumn - the grains (*Betulae semen*) (Brouneton, 1993; Pitera, 2000).

The birch tree is also called the nephritic tree of Europe, its leaves being known in popular medicine, from antique times as valuable diuretic remedies.

The chemical composition of the foliar buds comprises polyphenolic compounds, vegetal hormones, mineral salts, triterpenic structures (betulin, lupeol), essential oils; the leaves contain flavonoids and saponins, and the bark contains large amounts of triterpenes, especially betulin (Ciulei et al., 1993; Dehelean et al., 2006; Peev, 2007).

The birch sap is recommended as diuretic, anti-infectious, anti-rheumatic, anti-inflammatory remedy, administrated as 200-300 ml/day. The Xth French Pharmacopoeia suggests a stabilization of the sap with ethanol 96^{c} (birch sap: ethanol= 8:2) (Peev, 2007).

The present study concentrated on an organoleptic evaluation of the spring vegetal products of the birch tree. The birch sap and the foliar buds were analyzed on an animal model for the diuretic and uricosuric activity as well as a preliminary screening of the antiproliferative potential, applying the phytobiologic test on *Lepidium sativum* grains in order to establish possible correlations.

MATERIALS AND METHODS

The birch trees which were the source of the spring vegetal products were under evidence for sylvan use in the summer of 2007. The trees were located in Padurea Neagra, Apuseni Mountains, Bihor County, at 900-1000 m altitude. Identification of the species was done in collaboration with the local Silvic organization.

The procedure of Birch sap harvest. From the 13th to the 17th of April incisions were cut into the trunk of the trees, at 0.5-1.0 m from the ground, in several spots with the aid of a drill performing incisions of 4, 6 and 8 mm. Flexible plastic tubes were fixed in the holes permitting the sap to be collected in special flasks. At the end of the process wooden corks were inserted in the holes (Carole et al., 2007).

Density and dry residue determination were done in conformity with the 10th Romanian Pharmacopoeia monograph (FR X, 1993).

The procedure of harvest and formulation of birch tree foliar buds. 500 g of foliar buds were manually harvested at the beginning and at the middle of March, as well as at the beginning of April, right before their opening. The buds were dried in the shadow and grinded in a pharmaceutical mortar. The 6% aqueous extract was prepared with the aid of an ultrasound bath, for 15 minutes.

Water content of the birch foliar buds was determined following the Xth Romanian Pharmacopoeia monograph, in triplicate (FR X, 1993).

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Diuretic and uricosuric evaluation. The pharmacologic experiment was performed on 4 groups of white Wistar male rats, of 120-150 g each. The animals were kept under standard conditions. At the beginning of the experiment they were all uniformly hydrated through the administration of 2.5 ml of physiologic serum /100 g body weight. After 45 minutes the animals were administered intraperitoneally the following samples:
- group 1 = control group: 1 ml distilled water
- group 2: 1 ml solution of furosemid 30 mg/kg body weight
- group 3: 1 ml solution birch foliar buds extract 5%
- group 4: 1 ml birch sap

After 24 h from the administration time, each animal was placed on a diuresis device, the urine being collected in graded cylinders. The uric acid content was determined collorimetrically, at 670 nm, the results being expressed as mg/24h/kg body weight.

Phytobiologic test on *Lepidium sativum* grains. This *in vivo* test provides data regarding the inhibition of cellular multiplication, the mitodepresive, citostatic as well as stimulating cellular activity of natural compounds. The grains of *Lepidium sativum* are set to germinate in flasks with the diameter of 5 cm. After 24-48 h the radicules are being measured on milimetric paper both from the grains treated with the extractive solutions in several concentrations and those treated with water as control samples. For each sample we performed 5 measurments of 10 plantules. The inhibition rate is calculated with the formula:

$$I = \frac{Lm - Lt}{Lm} \times 100$$

where:
Lm = the average lengh of radicules from grains treated with water
Lt = the average lengh of radicules from grains treated with the extractive solutions

Values higher than 50% for the inhibition rate indicate a potential antiproliferative effect; the extractive solutions for which the results show this kind of activity, must be considered for further analysis in on superior level of the screening process (Peev, 2007).

RESULTS AND DISCUSSIONS

Birch sap is a clear liquid, transparent to slightly opalescent, having a sweetish taste and no odor. The speed of sap discharge was the same during the daytime and nighttime, 1.5-2 l of sap being collected in 12 h. The whole process of sap drainage took 20-21 days. The density of the sap was evaluated at 1.20 for the day sap and 1.05 for the sap collected during the night.

The length of foliar buds was evaluated at 4-5 mm, having ovoid, conical aspect and brownish dark green color. They are covered with a sticky resinous substance which confer them a shiny aspect.

The content in water was of 28.9% at the beginning of March, of 34.6% at the middle of March and of 53.6% at the beginning of April, before opening.

The values obtained for the diuretic effect and that of the uricosuric activity are presented in Table 1.

Table 1

Results of diuretic activity assay expressed as ml of urine, respective mg of uric acid eliminated of 1 kg body weight of animal per day

Samples	Diuresis (ml/kg/day)	Acid uric (mg/kg/day)
Control	19.23	0.23
Furosemid	38.13	1.42
Birch foliar bud extracts	18.23	1.32
Birch sap	27.81	2.91

The group treated with the extract of foliar buds produced a diuretic effect similar to the group treated only with water, but had a stronger uricosuric activity.

The birch sap had an intermediate effect on the diuretic process, between the control group and that treated with furosemid. The elimination of uric acid was significant, almost two times more intense than that determined by furosemid.

The results obtained for the inhibition of the germination of *Lepidium sativum* grains for the birch sap at different dilutions are presented in Fig. 1, while those obtained for foliar buds extracts are showed in Fig. 2.

The present study confirmed that the period needed for the collection of birch sap is that of 3 weeks. Before and after this period, the flow process of sap is stopped. Average speed of sap flow is constant during daytime and night-time.

In spring foliar buds know an increase in metabolic activity, recording a gradual accumulation of water, from 28% to 53%, with a difference of 25 percentage points between the lag phase and the phase before opening. Birch buds extracts are known in literature to possess an inhibitory action on cell development on cellular lines, in in vitro studies (Fulda, 2008; Pisha et al., 1995).

Birch sap has a strong diuretic action: 72.94%, demonstrated on animal model. Diuretic action of birch sap is higher than the extract of foliar buds (46.89%). Birch sap has also presented a uricosuric effect two times more intense than that caused by furosemid or by extract of birch foliar buds.

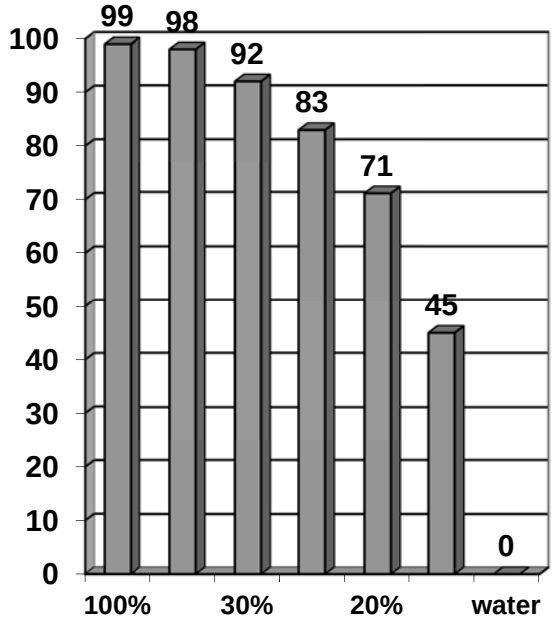

Fig. 1 Diagram of the inhibition rate induced by several dilutions of the birch sap

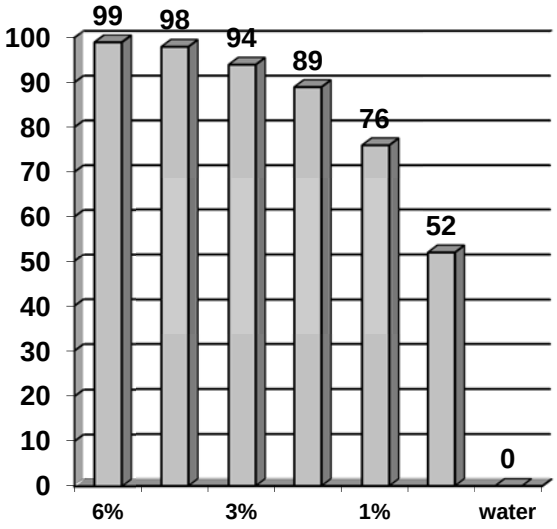

Fig. 2 Diagram of the inhibition rate induced by several dilutions of the birch foliar buds extracts

CONCLUSIONS

This information is important for future possible achievement of a birch trees culture as experimental lot with appropriate centralized system of sap collection.

The phytobiologic test showed favorable data concerning the ability of inhibition of cellular division, a possible antiproliferative activity, linked to the anti-tumor active compounds contained by the analyzed vegetal products. This preliminary study of birch sap correlates with multiple therapeutic recommendations in folk medicine (Peev et al., 2009). To elucidate the complex mechanism of action is necessary to establish an accurate chemical composition and to achieve pharmacotoxicological tests in vitro and in vivo.

The two findings may suggest lines of prevention and treatment of inflammatory, proliferative diseases, with possible tropism for those localized at the urinary system level.

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