Complexation with Hydroxypropyl-γ-Cyclodextrin of Birch Tree Extract
Physico-chemical Characterisation of their Binary Products

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Birch bark contains important pentacyclic triterpenes that determine an anticancer, anti-inflammatory and antiviral activity. The compounds can be extracted by simple procedures with organic solvents. The major problem of this type of triterpenes is their low water solubility which can be increased by physical procedures like cyclodextrin complexation. The aim of present study was to analyse the products between birch bark extract and hydroxypropyl-γ-cyclodextrin. Hydroxypropyl-γ-cyclodextrin (HPGCD) was used as a host to improve its solubility in water, via inclusion complex formation. In order to obtain the inclusion complexes, 1:2 molar ratio and two preparation methods (physical mixing, kneading) were used. The inclusion complexes were analyzed by in vitro dissolution tests, thermal analysis and X-ray diffraction.

Keywords: HPGCD, birch tree, inclusion complexation, solubility

Saponins are compounds with steroid or terpenic structure, widely distributed in the vegetal world and having an amphiphilic character [1]. Triterpenes like betulin, lupeol and especially betulinic acid, main components of birch bark, display anticancer activity [2]. Important triterpenes are found in the outer bark of the birch tree (Betula pendula Roth); of these compounds, betulin is the major one as its content surpasses 20% [3].

The studies cited above have been performed on pure compounds, however, plants contain complex mixtures of substances known to possess synergistic activities [4-6]. In this concept, the present study aimed at researching the antitumour activity of birch extracts rather than of pure substances.

Cyclodextrins are torus-shaped oligosaccharides, built up from glucopyranose units, obtained by fermentation of starch. Cyclodextrins are able to form inclusion complexes with a great number of compounds, which may improve the guest solubility, bioavailability, physical-chemical stability, both in solid state and in solution [7, 8].

The aim of this study is the formation of inclusion complexes between triterpenic compounds of birch tree extract (betulinic acid, betuline, lupeol) and hydroxypropyl-γ-cyclodextrin (HPGCD); by complexation, the triterpenic compounds are molecularly dispersed in a hydrophilic matrix and become soluble [9], which leads to faster dissolution and a better bioavailability. Products containing 1:2 molar ratio of BC1 : HPGCD , prepared by different methods, have been investigated by various techniques.

Experimental part

Hydroxypropyl-γ-cyclodextrin was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). It was used as received.

The plant material, outer bark of birch tree, Betula pendula Roth, (Betulaceae) was harvested from the Aninei Mountains (Banat region, South West of Romania) in October 2007. Voucher samples were deposited in the Herbarium of the Department of Pharmaceutical Botany of the Faculty of Pharmacy, University of Medicine and Pharmacy, Timisoara. Only the outer part of the bark (the cork) that spontaneously separates from the stem in autumn was used. The cork was air-dried at room temperature, broken in small pieces and ground to powder using a mill. All solvents were reagent grade. The extracts were prepared from Betula pendula Roth outer bark by Soxhlet extraction in methanol t in 2 h. The drying process was done using a Büchi rotavapor device. The etalons were purchase from Extrasynthese with over 96% purity.

Preliminary studies

In the pre-experiment studies, we recorded the absorption curve of BC1, the maximum of absorption (λ_max = 276 nm) and we established that the pH of the solution did not influence the profile of the spectrum.

Preparation of solid products

Different methods were applied in the preparation of the inclusion complexes:
- simple powder mixing, using a mortar and a pestle;
- kneading with a 50% ethanolic solution until the bulk of solvent evaporated; the mixture was then dried at room temperature for 24 h and then was put in the oven, at 105°C for several hours. The final product was pulverized and sieved.

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The binary products were prepared using 1:2 molar ratio, calculated on the basis of molecular weights of HPGCD and betulinic acid; the percentage of betulin in the birch tree extract was estimated to 40%.

In vitro dissolution studies
The dissolution studies were carried out by using a modified paddle Erweka DT apparatus, in 100 mL buffer solution of pH = 5.5, at 37±1°C, for 120 min. Samples were taken after 5, 10, 15, 30, 60 and 120 min and the dissolved quantity of BC1 was determined spectrophotometrically at 276 nm. All studies were done at least in triplicate.

Thermal analysis
BC1, HPGCD and their products were analyzed by thermogravimetry and differential thermal analysis, using a Mettler TGA/SDTA 851/LF/1100, in platinum device in 30 µL platinum creosotes, under nitrogen flux (150 mL/min.). Warming speed were 2 and 10 min respectively. The sample quantity was 4.502 mg.

Argon was used as carrier gas, the heating rate was 5°C / min and the sample weight was 2-5 mg. Examinations were made from 25°C up to 300°C.

X-ray diffraction analysis
XRD spectra were recorded with a DRONUM-1 X-ray diffractometer (Russia) system with CuKα1 radiation (λ = 1.54178 Å) over the interval 2-44°/2θ. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 35kV; current, 20 mA; time constant, 1S; angular range 2° < 2θ < 44°.

Results and discussions
In vitro dissolution tests
Aqueous solubility of BC1 is increased by HPGCD, which also influences the dissolution rate of the active substances. The physical mixtures prepared with HPGCD yielded a higher dissolution profile as compared with BC1 itself and the kneaded products were better than the physical mixtures. Figures 2-3 show the dissolution profiles for BC1 and its products with HPGCD in buffer solution.

Thermal analysis
The TG, DTG and DTA curves of BC1 and its 1:2 kneaded product with HPGCD are shown in figure 4. The TG curve for BC1 alone exhibits an endothermic peak around 350°C, due to the melting of the active compounds contained in the extract. HPGCD decomposes at a higher temperature than the one mentioned. For the kneaded product the TG curve exhibit an endothermic peak before 300°C probably corresponding to the decomposition of the entire sample. Beside the phase transition, the occurrence of a chemical reaction between the host and the uncomplexed guests cannot be excluded. The shift of the melting point toward a lower temperature can be interpreted as an interaction between the triterpenic compounds and HPGCD [11, 12].

X-ray diffraction
X-ray diffraction analysis confirms the thermal analysis results. As can be seen in figure 5 a, b BC1 presents some peaks, characteristic for crystalline compounds, whereas they are practically absent in the respective KP products. The disappearance of all crystalline peaks leads to the
conclusion that an amorphisation phenomenon takes place, which can be interpreted as an inclusion complex formation between BC1 and HPGCD.

**Conclusions**

HPGCD is able to form inclusion complexes with compounds that fit its cavity dimensions. The solubility of triterpenic compounds was increased in the presence of HPGCD.

Products were prepared in molecular ratio 1:2, using physical mixing and kneading method. The formation of inclusion complexes has been proved by thermal analysis.
studies, which confirmed the efficacy of the kneading method in this purpose.

In vitro dissolution tests revealed that the kneading method significantly improved the rate of dissolution, especially at a molar ratio of 1:2.

The presence of HPGCD significantly influences different parameters of the drug such as solubility and dissolution rate; this could prove advantageous in the future, offering the possibility of new pharmaceutical preparations with higher bioavailability and smaller therapeutical dosage.


References

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