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Isolation of triterpene-rich extracts from outer birch bark by hot water and alkaline pre-treatment or the appropriate choice of solvents

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ABSTRACT

Birch bark, left over as residue fuel in the pulp and plywood industry, is rich in pentacyclic lupane-type triterpenes – primarily betulin and lupeol – promising starting materials for the synthesis of biologically active compounds with a broad spectrum of medical applications. A comparative study on the main triterpenes of the outer bark of two birch species – silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.) – is reported. The total yield of extractives decreased with crop age. For the first time, pre-treatment with hot water and Na_2CO_3 water solution of birch outer bark was carried out before extraction. Pre-treatment with Na_2CO_3 water solution substantially improved the ethanol extracts' triterpene content (from 67.7 to 99.0%). The effect of different solvents on the yield and composition of extracts was studied. Nonpolar solvents behave more selectively toward triterpenes and admixtures. It is possible to improve the extracts' triterpene content by modifying the extraction technology employed and choosing the appropriate solvents.

1. Introduction

The primary birch species in the Northern hemisphere used for pulp and plywood production are silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.) (Bergelin et al., 2008). Birch bark comprises approximately 12.5% of the tree mass (Kislitsyn, 1994). Birch logs contain approximately 2.0–3.4% of outer bark (Kislitsyn, 1994; Pinto et al., 2009)—the region of bark spanning from the last formed periderm layer to the very outer surface of the tree (Gilberg, 1986). It is already known that the outer bark of most birch species, widespread in Northern Europe, is rich in pentacyclic lupane-type triterpenes—mainly betulin and lupeol, less betulinic acid, and others (Fig. 1) (Eckerman and Ekman, 1985; Kislitsyn, 1994).

These representatives of triterpenes possess known biological activity, which can be enhanced by synthetic modification to obtain compounds with interesting pharmacological properties and a wide application range (Alakurtti et al., 2006; Kessler et al., 2007; Tang et al., 2011). In addition to exhibiting promising therapeutic properties, betulin is an excellent water-oil system emulsifier for the production of cosmetic products, demonstrating a positive anti-inflammatory side effect (Weckesser et al., 2010). It has been well established that these triterpenes can be extracted in good yields (20–40%) from outer birch bark with a great number of different organic solvents (Eckerman and Ekman, 1985; Kislitsyn, 1994). Birch Kraft pulp mill, with an annual production of 4,00,000 ton/year, and a plywood factory, with an annual production of 2,52,000 m^3/year, generate approximately 28,000 and 16,000 ton of outer bark, respectively (Pinto et al., 2009). The calorific value of outer bark exceeds 30 MJ/kg; therefore, together with other residual materials (i.e., veneer shorts, woodchips and sawdust), it is incinerated in the power plants of pulp and plywood mills (Nurmesniemi et al., 2012). A comparison of available alternatives that could add value to forest industry waste reveals that, although the energy value of bark is approximately 20€ per ton (with the price of oil at 60 USD per barrel) (Hokkanen et al., 2012), the current sales price of one ton of betulin is 2500€ per kg (www.betulinines.com, 2015), which means that 1 ton of outer birch bark can provide up to 625,000€ of added value if the betulin yield is approximately 25%. The price of betulinic acid, which can be readily synthesized from betulin and lupeol (Kim et al., 1998; Chen et al., 2009), is even higher—8100€ per kg (www.betulinines.com, 2015). Therefore, this inexpensive but valuable natural material – birch outer bark – should be processed into products of higher added value than that of fuel. Additionally, after triterpene extraction, the remaining biomass can still be used for energy or for the production of other high-added-value products from suberin

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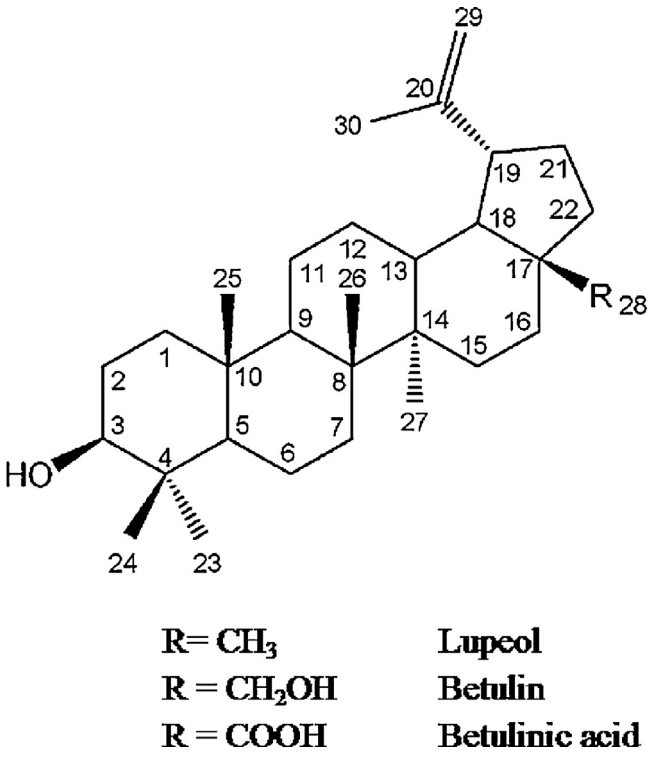

Fig. 1. Chemical structure of lupeol, betulin and betulinic acid.

(Gandini et al., 2006). Value is only one of the important factors for profitability. Another one is volume, when the industry should be able to produce high purity triterpenes from birch bark in great amounts using simple and environmentally friendly techniques, which are missing or the reported approaches are not good enough.

Although the history of betulin spans over more than two centuries and publications about triterpene isolation from birch outer bark and the crystallization of betulin date back to the first half of the 19th century (Hünefeld, 1836), researchers' interest in this chemical species has not diminished. The current number of scientific articles and patents concerning betulin and its derivatives is noteworthy (in 2014–770 articles, by "SciFinder"). Despite this continued interest and research activity, no pharmaceutical preparations have been developed and produced based on betulin and other triterpene components of outer birch bark. Although numerous highly promising areas of application have been established, progress has been reduced by the lack of the industrial availability of the triterpene starting material of interest. One of the reasons for this situation is supposed to be the lack of engineering data on the processing of birch bark. In this context, the present study was performed to comparatively examine the main triterpenic composition of the outer barks of silver and downy birch. In plywood manufacturing, to facilitate peeling, veneer logs should be conditioned to soften the wood and provide an acceptable quality of veneer. Conditioning involves the exposure of veneer logs to both heat and moisture by soaking in hot water tanks (16–18 h and 40–60 °C) (Shulga et al., 2012). This procedure serves as a hot water extraction process. Therefore, in this study, birch bark obtained from a plywood factory and that of freshly cut trunk were compared, and the effect of the solvent type and pre- treatment (i.e., via hot water or alkaline water solution) on the yield and variability of the betulin and lupeol contents of the obtained extracts was investigated. The main aim of the study was to obtain extracts with a high content of triterpenes by using environmentally friendly and less harmful methods, which could serve in industrial scale production. There are two ways to obtain such extracts without admixtures of phenolic compounds and monosaccharides: (1) discharging the aforementioned substances from outer birch bark by pre-treatment with hot water or Na_2CO_3 water solution and subsequently extracting the target species using less toxic polar solvents; or (2) using nonpolar solvents, such as petroleum ether, in which the admixtures are not soluble.

2. Materials and methods

2.1. Feedstock

Isolated birch bark, left over at a plywood factory in Latvia, was selected as representative industrial waste. Outer bark specimens were obtained from growing (crop age 15 years) silver birch (SB) and downy birch (DB) trees at 3 different heights (0–60, 60–130 and 130–210 cm), and specimens from freshly cut trunks with a crop age of 60 years were collected in forests of Latvia (coordinates: 57°45′18″N; 24°21′57″E) for comparison purposes.

The aforementioned birch bark samples, dried at room temperature (moisture content 10%), were milled in an SM 100 cutting mill (Retsch GmbH & Co.) to pass through a sieve with pores measuring 2 mm in diameter. Milled dry birch bark samples were soaked by mixing sporadically in deionized water for 24 h. Birch outer bark, which floated to the top of the water surface, was collected and dried to a moisture content of 2–4%. The inner bark sank to the bottom.

2.2. Identification of birch bark species

For the identification of downy and silver birch outer barks, a chemical indicator and the inner bark of the corresponding trees (Lundgren et al., 1995) were used. Diarylheptanoid glycoside platyphylloside, present at high levels in silver birch inner bark (20–30 mg g^{-1}) and at low levels in downy birch inner bark (≤0.5 mg g^{-1}), made it possible to distinguish between these species by using a 2,4-dinitrophenylhydrazine (2,4-DNFH) solution prepared from 100 mL 2 M HCl (Acros Organics, Belgium) + 0.4 g 2,4-DNFH (Acros Organics, Belgium). The indicator immediately formed an orange precipitate with platyphylloside.

2.3. Pre-treatment

Approximately 50 g of air-dried birch outer bark with known moisture content, obtained from a plywood factory, was placed in a 1000-mL conical flask, preliminarily weighed to an accuracy of 0.01 g and poured over 500 mL of distilled water, Na_2CO_3 (Acros Organics, Belgium) and NaOH (Acros Organics, Belgium) water solution (2.5 and 7.5% from birch outer bark). The mixture was heated over a silicone oil (Acros Organics, Belgium) bath (T = 110 °C) for 3 and 5 h by refluxing. The treated birch outer bark samples were dried for further extraction. The obtained water extracts were stored in closed test tubes at 4 °C in the dark for monosaccharide and phenolic compound content analysis.

2.4. Determination of monosaccharides in pre-treatment extracts

The monosaccharides (calculated as hexoses) in the extracts after the hot water or alkaline solution pre-treatment were determined by the Malaprade reaction, in which 1,2-diols and 1,2,3-triols were oxidized by potassium periodate (Acros Organics, Belgium), described by Besada and Gawargious (1974).