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Research paper

An approach to effective green extraction of triterpenoids from outer birch bark using ethyl acetate with extractant recycle

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ABSTRACT

The outer birch bark of *Betula pendula* Roth. was extracted using dry ethyl acetate, wet ethyl acetate (~3.5% H_2O) by the classical reflux boiling. The yields of the extracts, the content of triterpenoids and polar impurities were compared with the results of the extractions employing aqueous ethanol (5% H_2O). A higher content of betulin and a lower content of polar impurities were found for ethyl acetate extractions compared with the ethanol-based experiments. Alkaline washing of betulin concentrates was proposed as an alternative cost-effective method for purifying extracts of polar impurities. The uptake of residual miscella on the depleted birch bark was assessed to be up to 200–300% relative to the bark charge. An efficient recovery of the residual ethyl acetate by hydrodistillation from the extracted bark was demonstrated. An assessment of stepwise energy consumption for the extraction, concentration of extract, solvent recovery established that the extractions using ethyl acetate are significantly energy-saving, compared with ethanol usage. Batch extraction of birch bark employing ethyl acetate (96.5%) with hydrodistillation of residual solvent from raw materials can be used for a small-scale process to obtain betulin concentrates. According to the scheme proposed, complete recycling of the organic and aqueous phases is carried out and extractant losses are practically excluded. Considering large-scale processes, the continuous extraction scheme with solvent separate recovery from waste materials in the special equipment is feasible with dry solvent application. Extracting birch bark with "green" extractant ethyl acetate is beneficial, since it provides extracts containing less polar impurities and significantly reduces energy consumption and extractant losses comparing with use of polar extractants.

1. Introduction

Birch-tree bark, a large-scale timber-processing waste is a source of valuable extractive components, among which lupane-type triterpenoid betulin (up to 30%, w/w) is of major importance. Extraction of triterpenoids can be considered among the most prospective ways to solve the issue of bark waste processing.

Accessible concentrates of betulin are utilized as beauty ingredients and functional food additives (Krasutsky, 2006). Application of betulin as the starting compound for synthetic purposes is of particular interest. Synthetic lupane-type derivatives often manifest higher bioactivity levels, in particular, anti-cancer and anti-HIV, compared with betulin (Tolstikova et al., 2006). Numerous lupane derivatives were synthesized in the last decades (Cichewicz and Kouzi, 2003; Yogeeswari and Sriram, 2005).

The up-to-date modest level of betulin consumption may be partly related to technological imperfections (unsatisfactory economic performance and the environmental impact). Thus, developing novel, environmentally sustainable and energy-saving technologies of birch bark extraction seems promising.

Contemporary studies on birch bark extraction consider mainly efficiency of leaching the valuable components (betulin) and application of the "green" extractants. The most developed methods are based on water-soluble lower alcohols for betulin extraction (Drag et al., 2003; Şoica et al., 2012). Research findings on a large-scale scheme of birch bark processing with estimated solvent and energy consumption have been published recently (Fridén et al., 2016).

Special procedures have been developed to improve the betulin yield. Thus, in order to destruct a birch bark suberic matrix, preliminary treatment with steam or alkaline agents was proposed (Kuznetsova et al., 2014). Ultrasonic-assisted extraction (Qi-he et al., 2009) and supercritical extraction (Yu-hong et al., 2003) were also reported. On the other hand, certain important aspects of the extraction technology, in particular, selectivity, extractant recycle

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and energy-saving received less attention. For instance, recovery of residual solvent from the post-extraction plant material was scarcely reported. Extractant losses on depleted plant cake can contribute significantly to both economic and environmental impacts (Phennig et al., 2011).

Several studies on betulin extraction and purification gave reasonable preference to lower alcohols, especially, ethanol (EtOH) as an effective and "green" extractant (Fridén et al., 2016). Meanwhile, extraction of birch bark using lower alcohols has certain disadvantages in terms of energy use and solvent recycle. At the same time, application of intermediate polarity solvents for efficient retrieval of terpenoids from natural raw material was technically sound (Chemat et al., 2015). For example, ketones and esters can be considered. We do not regard halogenated solvents as potential extractants due to their high toxicity. "Green" solvent acetone has no advantages over EtOH for the extraction efficiency and product purity (Fridén et al., 2016). To promote extraction efficiency, selectivity and energy saving, we focused our study on the use of an alternative "green" solvent, ethyl acetate (EtOAc) (Prat et al., 2016; Alder et al., 2016). Certain data on the laboratory use of ethyl acetate to extract betulin from birch bark (*Betula platyphyla*) can be found in the literature (Zhao et al., 2007; Chue et al., 2011). Nevertheless, no research on environmentally sustainable bark extraction with EtOAc, including solvent recovery, is known to date.

The comparative literature data for "green" solvents EtOH and EtOAc, perspective as betulin extractants, are represented in Table 1.

Betulin is better soluble in EtOAc, as compared with the corresponding data for EtOH (95%) (Cao et al., 2007). However, EtOAc reflux boiling (RB) extraction of *B. platyphylla* bark provided betulin extract in somewhat inferior yield comparing with that for EtOH (95%) usage. The latter fact was explained in terms of co-extraction assistance by polar admixtures in the case of EtOH extraction (Zhao et al., 2007). At the same time, a high polarity of EtOH results in certain technical problems in the extraction process. Significant amounts of polar impurities are extracted with EtOH. Due to high heat expenses for evaporating EtOH or its mixtures with H_2O, concentration of extracts, removal of EtOH from depleted materials and regeneration of EtOH solutions, especially those containing much water, are extremely energy-consuming operations (Fridén et al., 2016). An incomplete recovery of ethanol from the extracted bark and from the stage of betulin purification can lead to solvent losses which can impair significantly both the economic and environmental parameters.

We assume that the use of a "green" aprotic solvent EtOAc, which has a significantly lower heat of evaporation, lower viscosity and low water solubility, can ensure a series of advantages: an improved selectivity of betulin leaching and solvent recycling and minimized energy consumption. Two approaches to implementing EtOAc extraction of birch bark, which can be different in a scale and methods of solvent removal from processed raw materials, are considered. First, a batch extraction of birch bark accompanied with recovery of EtOAc from the depleted cake by direct steam or hydrodistillation in the same extractor is feasible. In this case, steam or hot water may be used for solvent recuperation, while regeneration of EtOH by hydrodistillation from the depleted cake is technologically inappropriate.

The process of EtOAc azeotrope condensation forms two liquid phases: the organic phase (EtOAc with an equilibrium amount of dissolved water ~3.5%) and a small amount of the aqueous phase (EtOAc ~8.7%). Considering solvent recycling, drying of wet EtOAc (96.5%) resulting from the hydrodistillation seems hardly reasonable due to the insignificant water content. Therefore, we were interested in a study of bark extraction using water-containing EtOAc 96.5%. Second, extracting bark with dry hot EtOAc is possible. Special types of equipment for the continuous extraction and solvent regeneration are implemented in the industry at present (Phennig et al., 2011). In this case application of dry EtOAc is supposed to be advantageous due to a lower heat consumption compared with the extractions employing polar extractants.

The approaches to the extraction of betulin from the outer bark separated from the bast (Krasutsky et al., 2004; Rizhikovs et al., 2015) as well as from raw material without (bast) separation are known (Levdanskii et al., 2004). In spite of the additional costs of bark partition, separate extraction of outer bark seems to be a more reasonable approach for betulin production (especially at a small scale). Inner bark is the ballast material which is low in betulin and rich in hydrophilic metabolites contaminating the target component. Preliminary separation of inner and outer bark may reduce extractant and energy consumption significantly (both for extraction and solvent recovery), and simplify product purification. Outer and inner bark can be easily separated after grinding in order to facilitate sustainability (Rizhikovs et al., 2015). Therefore, we studied extraction of outer birch bark.

Thus, the research objective is to study birch outer bark extraction with "green" solvents EtOAc and aqueous EtOAc (96.5%), in comparison with EtOH (95%), determine the solvent recovery conditions and estimate energy consumption for environmentally sustainable technologies.

2. Material and methods

2.1. Plant material

The batches of birch (*Betula pendula Roth.*) outer bark were obtained for the comparison and production purposes from 3 different suppliers: Novosibirsk Region, Russia (coordinates:54°19′54″N; 84°20′51″E) – "Nov"(30 kg); Altay Republic, Russia (coordinates:51°56′32″N; 86°02′30″E) – "Alt"(80 kg); Tomsk Region, Russia (coordinates:56°28′18″N; 84°37′50″E) – "Tom"(110 kg).

The bark was collected on the places of woodcutting from freshly cut trees with a crop age 60 years. The raw material from the different suppliers was preliminary dried in at 40–50 °C and comminuted on a knife grinder (IYBG-2, NIOCh, Russia) to obtain approximately 0.3 × 2 cm pieces.

The averaged samples were prepared for each local milled raw material (small portions were taken from different places of different sacks and then mixed together to obtain ~600 g samples).

The averaged samples of each comminuted raw material were dried in a drying cabinet at 40–50 °C to residual moisture content 6.6 ± 1.3%.

2.2. Reagents and standards

Betulin, betulinic acid (> 98% purity), oleanolic acid (97% purity) and lupeol (94% purity) were purchased from Sigma Aldrich Chemie GmbH (Schnelldorf, Germany). Ursolic acid (95%) was obtained from Acros Organics (Belgium). Methyl ursolate (99% purity) was obtained from Carl ROTH, (Karlsruhe, Germany) and was also prepared from ursolic acid and purified as described in the work (Takeoka et al., 2000).

95% EtOH (SpirtMed, Russia), 99.5%+ EtOAc (Vekton, Russia), water bidistillate from BE-4 system (NV-LAB, Russia) were used for extraction.

For the analysis method LC–MS grade methanol from Vekton (Russia), 2-amino-2-(hydroxymethyl)-1,3-propanediol (99.5%) from Sigma Aldrich Chemie GmbH (Germany) were employed. Deuterochloroform for NMR analysis was obtained from Astrakhim (Russia), 1-chloro-2,4-dinitrobenzene (99%) was purchased from Sigma Aldrich Chemie GmbH (Schnelldorf, Germany).