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Essential oil in *Betula* spp. leaves naturally growing in Estonia

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ABSTRACT

The essential oils of the leaves of *Betula pendula* Roth., *Betula pubescens* Ehrh., *Betula humilis* Schrank and *Betula nana* L. (Betulaceae), and *B. pendula* buds were obtained using hydrodistillation and their chemical compositions were analyzed using gas chromatography and gas chromatography – mass spectrometry. The yield of essential oil was the highest (0.27%) in leaves of. *B. humilis* and the lowest in the leaves of *B. pendula* (0.11%), *B. pubescens* (0.05%) and *B. nana* (traces). Overall, 35–60 compounds were identified representing more than 92% of the total volume of the oils investigated. The bicyclical sesquiterpenoids found in the essential oils extracted from the leaves of *B. pendula*, *B. pubescens* and *B. humilis* and from the buds of *B. pendula* where α-betulenol (20.4–33.1%), α-betulenol acetate (5,6–28.9%), β-betulenal (5.5–6.2%) and β-betulenol (2.0–5.8%). The buds of *B. pendula* contained more birkenal, (E)-β-caryophyllene, δ-cadinene and caryophyllene oxide, but less α-betulenol acetate, β-betulenal and aliphatic compounds than the oils extracted from the leaves investigated. The oil composition of *B. humilis* and *B. nana* leaves was studied for the first time. The content of essential oil of *B. nana* leaves was much more varied: the oil contained less bicyclical sesquiterpenoids and more aliphatic compounds than the other *Betula* species investigated. The findings of our study could be used in chemosystematics of birch species, especially for *B. nana*.

1. Introduction

The roots of therapeutic use of leaves and other parts of different birch species (*Betula* spp., Betulaceae) and products of birch lead back to the ethnomedicine of ancient times. Different parts of birch are used mainly for treating urinary tract disorders, severe infections and inflammations (Demirci et al., 2004; Raal, 2010). In Soviet Union and Russia buds of birch are, according to the U.S.S.R. 11th State Pharmacopoeia (State Pharmacopoeia, 1987), widely used as a diuretic. Even more widespread is the use of leaves as a part of a treatment process. Nowadays the birch leaf is used as traditional herbal medicinal product that increases the amount of urine and helps flushing the urinary tract, by acting as an adjuvant in minor urinary complaints (Raal, 2010).

According to the 7th European Pharmacopoeia (European Pharmacopoeia, 2010), the whole or fragmented dried leaves of *Betula pendula* and/or *Betula pubescens* as well as the leaves of hybrids of both of the species can be efficiently used for medical purposes. The leaves contain not less than 1.5% of flavonoids, calculated as hyperoside, with reference to the dried drug.

Birch has been considered the most complex genus of all circumpolar genera because of its frequent hybridisation and high morphological variability. For example, the separation between *B. pendula* and *B. pubescens* may be problematic because some individual trees show morphological characters that are intermediate between these two species (Lahtinen et al., 2006).

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Such morphological problems could be solved by studying the chemical composition of birch leaves. The latter chemotaxonomic studies have been based mainly on the composition of phenolics and terpenoids in birch leaves and other organs (Valkama et al., 2003).

The chemical composition of flavonoids, the main constituents of birch leaves, has been investigated quite extensively (Raal, 2010). The content and composition of essential oil of birch buds and leaves has not been studied as extensively (Soden and Elze, 1905; Vedernikov and Roschin, 1988; Vershnyak and Stepen, 1992; Nguyen et al., 1995; Galgon et al., 1999; Baser, 2000; Demirci et al., 2000a, b, c, 2004; Shabanova et al., 2002; Demirci and Baser, 2003; Isidorov et al., 2004; Klika et al., 2004; Vuorinen et al., 2005; Domrachev and Tkachev, 2006; Baser and Demirci, 2007; Vedernikov and Roshchin, 2009). The study of essential oil extracted from birch buds by hydrodistillation began more than 100 years ago (Soden and Elze, 1905), but the content of essential oil distilled from birch leaves has only been analysed in very few papers (Demirci et al., 2000c; Baser and Demirci 2007).

In Europe more than 65 species of *Betula* are known. They are grown mainly in the northern hemisphere and from Eastern Europe to the northern parts of Asia (Raal, 2010; Demirci et al., 2004). In Estonia there are four naturally growing birch species: silver birch (*B. pendula* Roth, syn. *Betula verrucosa* Ehrh.), white birch (*B. pubescens* Ehrh.), Arctic dwarf birch (*Betula humilis* Schrank, syn. *Betula fruticosa* Pall.) and dwarf birch (*Betula nana* L.) (Kukk and Kull, 2005). We did not found information about the essential oil composition of *B. humilis* and *B. nana*. In our study the essential oil content of mentioned birch species leaves was analysed for the first time.

2. Experimental

2.1. Plant material

Buds of *B. pendula* were collected in April from Tallinn, Harju County. All leaves were collected in the middle of June: *B. pendula* and *B. pubescens* from Tallinn, Harjumaa County, *B. humilis* from Risti bog, Lääne County and *B. nana* from Viru bog, Harju County. The plant organs were dried in a dark room at room temperature (20 ± 2 °C) for ten days. Each dried organ of a plant was labelled, packed in paper-bag, and stored in the dark at room temperature until assayed. Voucher specimens (No Betulaceae/Bet1-4) have been deposited at the Institute of Pharmacy, University of Tartu, Estonia.

2.2. Hydrodistillation

The air-dried birch leaves and buds were crushed and sieved (3.0 mm aperture). Before distillation the dried plant organs were tested to determine the amount of loss of drying (European Pharmacopoeia, 2010). The plant material (20 g) was hydrodistilled for 2 h using Ginsburg-tube described in (State Pharmacopoeia, 1987). The essential oil yields were calculated on a dry weight basis (State Pharmacopoeia, 1987).

2.3. Analysis of essential oils

2.3.1. Capillary gas chromatography (GC-FID)

Two fused silica capillary columns with bonded stationary phases (30 m × 0.25 mm, Supelco): poly(5%-diphenyl-95%-dimethyl)siloxane (SPB-5) and polyethylene glycol (SW-10). GC analysis is described in our previous investigations (Raal et al., 2008).

The components of oil were identified by comparing their retention indices (RI) on two columns to the RI values of reference standards, to our RI data and to literature data (Davies, 1990; Zenkevich, 1996, 1997; 1999). The results obtained were confirmed by GC-MS. Several compounds were identified tentatively by RI data from (Demirci and Baser, 2003; Demirci et al., 2004).

The percentage composition of the oils was calculated using normalization method without correction factors. The relative standard deviation of the percentages of oil components in three repeated GC analysis of the single oil sample did not exceed 5%.

2.3.2. Gas chromatography/mass spectrometry (GC-MS)

GC-MS analysis was carried out using a GC-MS-QP2010 (Shimadzu, Japan) on a fused silica capillary column (30 m × 0.32 mm) with a bonded stationary phase: poly(5%-diphenyl-95%-dimethyl)siloxane (ZB-5, Zebron). More details were described in (Raal et al., 2010).

3. Results and discussion

The yield of essential oils was the highest (0.27%) in the leaves of. *B. humilis*, the leaves of *B. pendula* (0.11%) and *B. pubescens* (0.05%) contained much smaller amounts of oil. As reported earlier (Lavrjonov and Lavrjonova, 1999), the essential oil content of *B. pendula* leaves was only 0.04–0.05%, which was similar to the essential oil content of buds. According to Vershnyak and Stepen (1992), only the buds of *B. pendula* afforded a good yield (3–7%) of essential oil; branches gave 0.2–0.3% and bark 0.01% of oil. According to the U.S.S.R. 11th State Pharmacopoeia, the essential oil content of birch buds should not be