**Original Article**

**Essential Oil of *Betula pendula* Roth. Buds**

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The essential oil of *Betula pendula* Roth. buds was obtained using both hydrodistillation and microdistillation techniques and their chemical compositions were analyzed using both gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Overall, more than 50 compounds were identified representing 80% and 92% for hydrodistillation and microdistillation, respectively. The main components (by hydrodistillation and microdistillation, respectively) found were α-copaene (12% and 10%), germacrene D (11% and 18%) and δ-cadinene (11% and 15%) in the analyzed essential oils. The microdistillation technique proved to be a useful tool and compliant alternative when compared to hydrodistillation.

**Keywords:** birch – *Betula pendula* – Betulaceae – essential oil – GC – GC-MS – hydrodistillation – microdistillation

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**Introduction**

Medicinal and aromatic plants have been used for many centuries and are still popular in today’s alternative therapies in first order. Throughout man’s history, almost every part of different *Betula* species has found various medicinal applications, which in part is due to their essential oils, depending on where they grow. *Betula pendula* Roth., also well known as the European white birch or silver birch, is one of the most common species of this genus. *Betula*, of the family Betulaceae, commonly known as birch, grow mainly in the northern hemisphere and from Eastern Europe to the northern parts of China and Japan almost all over the world. In Europe more than 65 *Betula* species are known (1,2).

Birch tree parts and products have been important since ancient times and have been used in traditional medicine in various forms, i.e. herb, whole extract, tar, oil, infusion etc. (3–5). The medicinal parts are regarded as the bark, leaves, buds, sap or juice or their processed products and are used to treat diseases such as urinary tract disorders, skin diseases, severe infections and inflammations. A herbal tea made from the leaves is recommended in Germany as a diuretic and for special diets. Furthermore, *B. pendula* flavors are used commercially as an aroma and flavoring for alcoholic beverages such as rum and non-alcoholic beverages such as root beer and other drinks. Due to the characteristic pleasant fragrance, birch products such as essential oils have also found many applications in cosmetics and related personal care products (3–10). Diverse phytochemical investigations of *Betula* species have shown that they contain mainly phenolics, flavonoids, tannins, saponins, glycosides, sterols and terpene derivatives (9–18).

Medicinal and aromatic plants are widely used today in modern phytotherapy. Birch tree products have an important place both in therapy and other products, in part due to their aromatic character. The essential oil composition can be a characteristic feature of a particular aroma or, more important, of a particular pharmacological activity. Although earlier studies on the essential oil chemistry of *Betula* sp. exist (10–18), this study mainly contributes to the essential oil analysis of *B. pendula* buds of German origin (which is an essential prerequisite for it’s subsequent medical application).

Both hydrodistillation and microdistillation (19–21) techniques were used to obtain the essential oil, which was subsequently subjected to gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).
Materials and Methods

Plant Material

Buds were collected from Maxhütte, Regensburg in April 2002. The plant material was air dried in a shady and aerated room until the weight was stable.

Isolation of the Essential Oil

The air dried buds were crushed using a mortar and were subjected to both hydrodistillation and microdistillation.

Hydrodistillation

The plant material was hydrodistilled for 3 h using a Clevenger type apparatus. The essential oil yield was calculated on dry weight basis corresponding to 0.5% (v/w).

Microdistillation

The plant material (~200 mg) was placed in the sample vial of the MicroDistiller® (Eppendorf, Germany) system together with 10 ml of distilled water. NaCl (2.5 g) and water (0.5 ml) were added into the collection vial to break any possible emulsion formation. Hexane (300 μl) was also added into the collecting vial to trap the volatile components. The sample vial was heated to 100°C at a rate of 20°C/min and then kept at 100°C for 15 min. It was then heated to 112°C at a rate of 20°C/min and kept at this temperature for 35 min. Later, the sample was subjected to post-run for 2 min under the same conditions. The collecting vial was cooled to –5°C during the distillation. After the distillation was completed the n-hexane-trapped volatiles were analyzed by both by GC and GC–MS.

Analysis of the Essential Oils

Gas chromatography

The oils were analyzed by GC using a Hewlett Packard 6890 system. An HP-Innowax FSC column (60 m × 0.25 mm, with 0.25 μm film thickness) was used with nitrogen as carrier gas (1 ml/min). The oven temperature was kept at 60°C for 10 min and programmed to reach 220°C at a rate of 4°C/min, then kept constant at 220°C for 10 min before proceeding to 240°C at a rate of 1°C/min. The split ratio was adjusted to 50:1. The injector temperature was set at 250°C. The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID), also set at 250°C.

Gas chromatography–mass spectrometry

The essential oils were analysed by GC-MS using a Hewlett-Packard GCD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (1 ml/min). GC oven temperature and conditions were as described above. The injector temperature was at 250°C. Mass

Table 1. Composition of essential oil Betula pendula buds

| Compound                              | RRI  | Products obtained by* |
|---------------------------------------|------|-----------------------|
|                                       |      | Hydro-               | Micro- |
|                                       |      | distillation         | distillation |
| Hexanal                               | 1093 | 0.1                   | 0.2 |
| 2-Pentyl furan                       | 1244 | tr                    | tr |
| p-Cymene                             | 1280 | tr                    | tr |
| 6-Methyl-5-hepten-2-one              | 1348 | tr                    | tr |
| Hexanol                              | 1360 | tr                    | tr |
| Nonanal                               | 1400 | 0.9                   | tr |
| *trans*-Linalool oxide (Furanoid)     | 1450 | tr                    | tr |
| α-Cubebene                           | 1466 | 0.8                   | 0.5 |
| cis-Linalool oxide (Furanoid)         | 1478 | -                     | 0.3 |
| α-Ylangene                           | 1493 | 1.1                   | 0.7 |
| α-Copaene                            | 1497 | 11.8                  | 9.6 |
| β-Bourbonene                         | 1535 | -                     | 0.1 |
| β-Cubebene                           | 1549 | 0.7                   | 0.5 |
| β-Ylangene                           | 1589 | 1.3                   | 0.4 |
| β-Copaene                            | 1597 | 1.0                   | 0.5 |
| β-Caryophyllene                      | 1612 | 3.4                   | 3.2 |
| 6,9-Guaiadiene                       | 1617 | 2.4                   | 1.9 |
| Aromadendrene                        | 1628 | 0.6                   | 0.3 |
| Cadina-3,5-diene                     | 1638 | 0.3                   | 0.2 |
| Alloaromadendrene                    | 1661 | 2.2                   | 2.2 |
| *epi*-Zonarene                       | 1677 | 0.6                   | 0.6 |
| α-Humulene                           | 1687 | 2.9                   | 3.0 |
| γ-Muurolene                          | 1704 | 2.6                   | 3.0 |
| Germacrene D                         | 1726 | 11.4                  | 18.0 |
| α-Muurolene                          | 1740 | 2.0                   | 2.5 |
| Carvone                              | 1751 | 0.3                   | 2.5 |
| δ-Cadinene                           | 1773 | 10.8                  | 15.3 |
| γ-Cadinene                           | 1776 | 2.4                   | 4.0 |
| Cadina-1,4-diene                     | 1799 | 0.3                   | 0.3 |
| 3,7-Guaiadiene                       | 1810 | 0.5                   | 0.7 |
| *(E,E*)-2,4-Decadienal                | 1827 | 0.2                   | tr |
| Calamene                             | 1849 | 0.3                   | 0.4 |
| Geraniol                             | 1857 | tr                    | 0.4 |
| *(E*)-Geranyl acetone                | 1868 | 0.1                   | 0.4 |
| *epi*-Cubebol                        | 1900 | 0.2                   | 0.3 |
| α-Calacorene                         | 1941 | 0.7                   | 0.5 |
| 1,5-Epoxy-salvial(4)14-ene           | 1945 | tr                    | tr |
| Cubebol                              | 1957 | 0.3                   | 0.4 |
| Dodecanol                            | 1973 | 0.1                   | 0.2 |
| γ-Calacorene                         | 1984 | tr                    | 0.2 |
| Caryophyllene oxide                  | 2008 | 0.5                   | 0.7 |
| Salvial-4(14)-en-1-one               | 2037 | 0.4                   | 0.4 |
| Humulene epoxide-II                  | 2071 | 0.5                   | 0.6 |
| Cubebol                              | 2080 | 2.7                   | 0.6 |
| 1-epi-Cubenol (n.p.)                 | 2088 | 5.0                   | 1.4 |
| Europelargone B                      | 2109 | 0.9                   | 1.4 |
| T-Cadinol                            | 2187 | 1.5                   | 3.4 |
Table 1. Continued

| Compound          | RRI  | Products obtained by*                  | Hydrodistillation | Microdistillation |
|-------------------|------|----------------------------------------|-------------------|-------------------|
| T-Muurolol        | 2209 |                                        | 0.9               | 1.7               |
| δ-Cadinol         | 2219 |                                        | 0.4               | 0.7               |
| α-Cadinol         | 2255 |                                        | 2.8               | 5.8               |
| Guai-6,10(14)-dien-4β-ol | 2269 |                                        | 0.1               | 0.4               |
| Tricosane         | 2300 |                                        | 0.5               | 0.3               |
| Eudesma-4(15),7-dien-4β-ol | 2369 |                                        | 0.1               | 0.7               |
| Pentacosane       | 2500 |                                        | 1.6               | 2.8               |
| Heptacosane       | 2700 |                                        | 0.1               | 1.1               |
| Total             | 80.1 |                                        | 92.4              |                   |

*aPercentages calculated from flame ionization detection data. RRI, relative retention indices calculated against n-alkanes; tr, trace amount (<0.1%); n.p., not pure.

spectra were recorded at 70 eV. Mass range was from m/z 35 to 425. Library search was carried out using the commercial resources Wiley GC/MS Library, MassFinder and the in-house Basér Library of Essential Oil Constituents.

Results and Discussion

The air dried crushed buds of B. pendula were utilized to obtain volatiles by means of hydrodistillation and microdistillation. The essential oils were further analyzed both by GC and GC–MS systems using a polar column, resulting in the identification of 50 constituents in the hydrodistilled sample, representing 80% of the total essential oil. The essential oil yield was 0.5% (v/w) in this case, low when compared to other previously investigated samples (15–18). In total, 53 constituents were identified in the microdistilled sample, representing 92% of the oil as seen in Table 1. Overall, sesquiterpene hydrocarbons were found in both samples as the dominating group of compounds. α-Copaene (12% and 10%), germacrene D (11% and 18%) and δ-cadinene (11% and 15%) were identified as the main constituents for the hydrodistilled and microdistilled samples, respectively. However, comparison of both samples suggested that both techniques resulted in a similar composition of the volatiles obtained. In this study, the essential oil profile of B. pendula obtained from the buds was quite different from that of previous investigations and results (15,17,18). In earlier studies betulenols were found to be the major constituents in the volatile oil of B. pendula (15,17,18,22). Kaneko et al. (10) also reported δ-cadinene (9.6%) as the main constituent of B. pendula from Japan.

Microdistillation was utilized here as a useful method enabling the analysis of ~200 mg samples of plant material for its volatiles compared to the hydrodistilled essential oil. This technique has also been successfully applied to analyze medicinal and aromatic plants in small quantities (20,21).

As an overall result, we can deduce that the microdistillation method is an efficient and practical technique, which can be used in the analysis of volatiles of various aromatic materials in a short time at less expense. However, this work also shows that further investigations on the essential oil and the evaluation of the biological activities of Betula species should be initiated. As a renewable bioresource, Betula species can serve as a good source for natural medicines and cosmetics with a traditional background.

Acknowledgment

The authors would like to thank Emeritus Prof. Dr R. Schönfelder, University of Regensburg, for helping in the identification of the plant material.

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Received December 4, 2003; accepted June 10, 2004