Studies on *Betula* essential oils

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Dedicated to Professor Atta-Ur-Rahman on the occasion of his 65th birthday

Abstract

Essential oils were obtained from leaf, branch and buds of *Betula* species: *B. pendula* Roth, *B. browicziana* A.Güner, *B. litwinowii* Doluch., *B. recurvata* V. Vassil., and *B. medwediewii* Regel naturally growing in various parts of Turkey. Also buds of the common birch *B. pendula* essential oil from Germany and two species native to Finland namely, *Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti and *Betula pubescens* ssp. *pubescens* Erhr. were investigated. *Betula* essential oils were obtained by different distillation techniques such as hydrodistillation, microdistillation and Likens-Nickerson simultaneous distillation-extraction method (SDE). The resulting volatile compositions were elucidated by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems.

Known and new sesquiterpenes were isolated from *Betula* essential oils using column chromatographic techniques. Structure determination of each isolated compound was carried out using 1D and 2D NMR spectroscopic techniques supported by MS, UV and GC FITR.

Biological activities were determined both for essential oils and pure compounds isolated from the oils of *Betula* species. Antifungal, antibacterial and antioxidant activity results were carried out using various *in vitro* techniques.

Keywords: Betulaceae, *Betula* species, Birch tree, essential oil, sesquiterpenes, caryophyllene, chromato-spectral techniques, biological activity

Introduction

Well-known as birch tree, the genus *Betula* of the family Betulaceae, has a wide distribution in the northern hemisphere from Canada to Japan.[1] Five *Betula* species, namely *B. browicziana* A. Güner, *B. litwinowii* Doluch., *B. medwediewii* Regel, *B. pendula* Roth and *B. recurvata* V. Vassil are naturally growing in eastern and northern Turkey, at high altitudes. Only *B. browicziana* is endemic to Turkey.[2,3]

The Birch tree has a long history of medicinal use in different countries and cultures to cure skin diseases especially eczema, infections, inflammations, rheumatism and urinary disorders.

Betula bud oil is also widely used in cosmetic products as a tonic and antiseptic mainly in hair products.[4-7]

Birch bark contains betulin, betulinol and a betuloside. The young leaves are rich in saponins and contain a diuretic flavonoid derivative (hyperoside), sesquiterpenes and tannins. The buds are rich in volatile oil. Birch tar contains creosol and guaiacol.[5,8]

The essential oils obtained from *Betula* species have been the subject of many investigations.[7-12] Betulenol, the main component of the oil, was isolated and reported from *Betula* buds and named first as betulol by Soden and Elze in 1905. Its structure was tentatively elucidated as a bicyclic primary sesquiterpene alcohol. Further investigations on this molecule were conducted by Triebs and other researchers as compiled in a work of Guenther.[5] Afterwards Treibs and Lossner reported α- and β-betulenol, their acetates and α-betulenal by means of synthesis to support the chemical structures present in the essential oil of *Betula lenta*.[8] In contrast, Holub reported the occurrence of α- and β-betulenol, as well as α-betulenol acetate with different structures as the previous investigators, also isolated from *Betula* species.[9] Dhar *et al*. reviewed the chemistry of the birch tree including the essential oil which appeared to support Holubs' previous work.[10] Hiltunen and co-workers reconfirmed by means of chemical reactions and gas chromatography / mass spectrometry (GC - MS), the occurrence of the main compounds as α- and β-betulenol and their relevant acetates in the bud oil of *B. pubescens* Ehrh., supporting Treibs' work.[11]

Essential oil components of *B. pendula* were analyzed by Stepen and co-workers using GC where the main components were identified as α-betulenol acetate, caryophyllene and derivatives including low amounts of α- and β-betulenol.[12] Kaneko *et al*. reported betulenols and their acetates in the essential oils isolated from the buds of nineteen *Betula* species.[7] Studies on the essential oils and sesquiterpenes of *Betula* species growing in Turkey were subject to several studies by our group.[13-18]

This present work covers the essential oil chemistry as well as biological activities of the main components isolated from various *Betula* species investigated by our group.

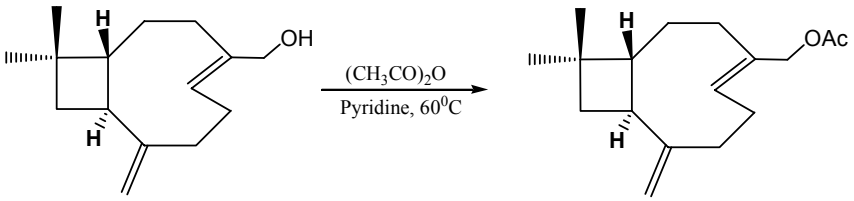
Results and Discussion

Caryophyllene and its derivatives have been of special interest in the field of natural products chemistry and subjected to many detailed works and reviews.[19-22] There have been many conflicts and disagreements concerning absolute configurations and structures of caryophyllene derivatives isolated from natural sources.[7-9,23]

The buds of *B. pendula*, *B. litwinowii* and *B. medwediewii* collected from various parts of Turkey were hydrodistilled while *B. browicziana* and *B. recurvata* buds were subjected to simultaneous distillation-extraction method (SDE) using a Likens-Nickerson apparatus due to limited plant material. The bud oils were analysed by GC-MS. The main component was isolated by Medium Pressure Liquid Chromatography (MPLC) in high purity.[13,16] Literature search and comparison with spectral data[23] confirmed the identity of this compound as 14-hydroxy-β-caryophyllene (**1**), which was found 20.5-37.5% in all investigated oils.

In the light of the recent accumulated data, we propose that 14-hydroxy-β-caryophyllene is synonymous with α-betulenol formerly isolated by Treibs from the bud essential oil of *B. lenta* [8]. The isolation of 14-hydroxy-β-caryophyllene (**1**) was also reported by Macleod[24] from *Pterocaulon serrulatum* (Montr.) Guillaumin, an Australian plant, supporting the previously reported data.[23,25]

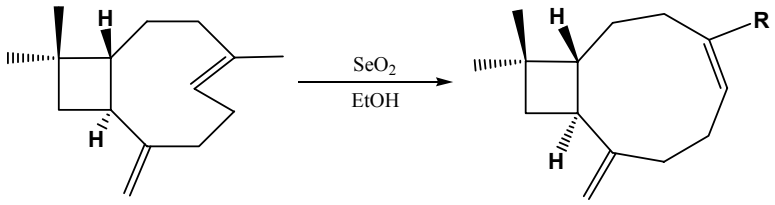
We acetylated **1** to form 14-acetoxy-β-caryophyllene (**1a**),[13] which was also shown to be naturally present in *Betula* bud essential oils (0.1-0.8%) by GC-MS (Scheme 1). Consequently, we suggest that 14-acetoxy-β-caryophyllene (**1a**) is synonymous with α-betulenol acetate, which was reported from *B. lenta* essential oil earlier.[8]



1 **1a**

Scheme 1. Acetylation of 14-hydroxy-β-caryophyllene (**1**).

β-Betulenal (**2**) which was isolated by MPLC from *Betula* essential oils was also synthesized. [13] β-caryophyllene (**3**) was treated with SeO_2, resulting in the formation of β-betulenal (**2**), 14-hydroxy-isocaryophyllene (**4**), and isocaryophyllene (**5**), as seen in Scheme 2. Compound **2** and **4** were isolated individually from the reaction mixture followed by structures confirmation by ^1H and ^{13}C NMR. This information supported that 14-hydroxy-isocaryophyllene (**4**) is synonymous with β-betulenol and β-betulenal **(2)** with isocaryophyllen-14-al, when compared with previous investigations.[8] To ensure the proposal, **2** was subjected to a mild reduction with $NaBH_4$, resulting in β-betulenol (**4**). These compounds were also detected in the *Betula* bud essential oils by GC-MS. Content of β-betulenol and β-betulenal, in the oils of investigated *Betula* species were found from trace to 1.2% and 2.0-5.2%, respectively (Table 1).



3

2 R= CHO
4 R= CH_2OH
5 R= CH_3

Scheme 2. Oxidation of β-caryophyllene (**3**).

Table 1. Main components of the different parts of *Betula* species growing in Turkey

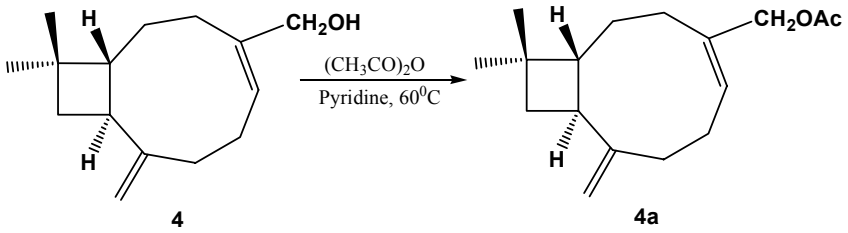
RRI	Compound	*Betula pendula* %			*Betula browicziana* %			*Betula litwinowii* %			*Betula medwediewii* %			*Betula recurvata* %		
		Br	L	B	Br	L	B	Br	L	B	Br	L	B	Br	L	B
1093	Hexanal	0.1	0.1	tr	0.1	0.1	tr	-	-	-	0.1	0.5	tr	-	0.1	-
1225	(Z)-3-Hexenal	tr	0.2	tr	-	0.4	tr	-	tr	-	-	5.8	-	-	0.4	-
1360	Hexanol	0.1	0.1	tr	0.1	0.1	tr	-	0.2	-	-	1.4	-	-	0.2	-
1400	Nonanal	0.2	0.1	tr	0.2	0.6	0.1	-	0.1	-	0.2	1.8	0.1	tr	0.2	-
1412	(E)-2-Hexenol	-	-	-	-	-	-	-	-	-	-	0.7	-	-	0.1	-
1553	Linalool	0.4	0.1	tr	0.3	0.5	0.1	0.5	1.0	-	0.3	2.8	tr	0.2	0.2	-
1612	β-Caryophyllene (**3**)	2.9	1.4	3.9	2.4	0.3	4.9	0.8	1.1	1.3	0.1	0.4	1.2	0.2	1.3	3.2
1687	α-Humulene	4.3	2.0	6.8	3.8	0.3	3.7	0.8	0.7	1.6	0.2	0.2	2.3	0.3	1.7	5.6
1706	α-Terpineol	0.1	tr	-	0.1	0.1	-	0.2	0.3	-	0.1	0.5	-	tr	0.1	-
1758	(E,E)-α-Farnesene	-	-	-	-	-	tr	0.2	0.6	-	-	1.9	tr	tr	0.1	-
1772	Citronellol	0.2	tr	tr	0.1	0.1	tr	0.3	0.7	-	0.6	-	0.1	0.1	0.1	-
1798	Methyl salicylate	tr	-	-	-	-	-	-	-	-	67.8	49.8	0.3	-	-	-
1802	Cumin aldehyde	-	-	-	-	0.8	-	-	-	-	-	-	tr	-	-	-
1804	Myrtenol	0.2	-	-	0.7	-	-	-	-	-	-	-	-	tr	tr	-
1834	ethyl salicylate	-	-	-	-	-	-	-	-	-	4.8	0.2	-	-	-	-
1857	Geraniol	0.3	0.1	0.1	0.2	1.1	tr	0.4	2.0	tr	3.4	1.8	0.4	0.1	0.6	tr
1958	(E)-β-Ionone	-	0.6	-	0.1	0.3	-	-	0.2	-	0.2	1.0	-	-	tr	-
2008	Caryophyllene oxide	4.0	4.3	5.3	3.9	2.3	6.1	2.3	3.1	3.2	0.4	0.5	2.6	1.6	2.9	1.7
2020	*des*-4-Methyl-caryophyll-8(14)-en-5-one (**10**)	5.3	4.7	5.1	6.9	10.2	5.2	3.1	5.7	6.0	2.2	0.8	7.8	4.2	6.9	3.9
2041	Pentadecanal	-	-	-	-	-	-	-	-	-	0.5	0.2	0.3	-	-	-
2045	Humulene epoxide-I	0.5	0.3	0.6	0.6	0.2	0.3	tr	0.1	0.2	-	-	0.2	0.1	0.2	0.2
2071	Humulene epoxide-II	4.9	4.8	6.9	3.9	2.4	4.2	1.4	1.5	3.3	0.4	0.4	3.1	1.3	2.6	2.4
2092	4,5-Dihydro-β-caryophyllene-14-al (**9**)	1.5	2.3	1.5	0.8	1.1	1.3	0.2	0.6	2.2	0.3	0.1	2.7	0.3	2.2	0.8
2100	Heneicosane	0.6	0.1	0.4	-	-	tr	0.3	0.3	-	0.1	-	0.4	0.4	-	-
2186	Eugenol	1.7	0.2	0.2	0.9	0.2	0.3	-	-	0.1	1.0	0.9	0.1	-	0.6	tr
2193	β-Betulenal (**2**)	7.6	4.7	4.0	11.1	13.9	5.2	5.5	7.3	3.3	0.7	0.8	2.7	4.4	5.2	2.0
2200	Docosane	0.1	-	-	-	-	-	0.2	1.1	-	-	-	-	-	-	-
2239	Carvacrol	-	tr	-	2.6	2.5	0.1	0.2	0.1	tr	2.2	0.6	1.0	0.2	0.1	tr
2272	14-Acetoxy-β-caryophyllene (=α-Betulenol acetate) (**1a**)	0.6	0.6	0.8	1.2	0.5	0.8	0.7	1.0	0.2	tr	-	0.1	3.7	2.3	0.5
2282	14-Acetoxy-4,5-dihydro-β-caryophyllene (**8a**)	0.3	0.2	0.4	-	0.2	0.3	0.3	0.4	0.2	0.1	-	0.3	0.5	0.3	0.3
2300	Tricosane	1.0	-	-	0.3	-	-	3.0	1.1	-	0.2	0.1	0.9	2.6	-	-

2316	Caryophylla-4(14),8(15)-dien-5β-ol (=Caryophylladienol I)	0.4	0.6	0.4	0.5	0.8	1.1	0.4	0.7	0.4	-	0.4	0.2	0.3	0.7	0.2
2324	Caryophylla-4(14),8(15)-dien-5α-ol (=Caryophylladienol II)	1.4	2.1	1.7	1.5	1.8	2.8	1.5	2.6	0.9	tr	-	0.6	1.0	2.1	0.6
2329	14-Acetoxy-α-humulene	0.3	0.2	0.3	-	-	-	0.2	0.2	0.1	-	-	0.1	0.2	0.1	0.1
2346	6-Hydroxycaryophyllene (**14**)	1.0	1.6	1.4	0.5	0.3	2.0	2.3	3.7	0.9	-	-	0.9	0.3	1.1	2.1
2357	14-Hydroxy-β-caryophyllene (=α-Betulenol) (**1**)	19.8	29.3	25.3	18.0	12.7	28.2	14.4	13.2	21.9	1.8	3.5	20.5	8.2	20.8	37.5
2384	Hexadecanol	-	-	-	-	1.3	-	-	0.3	-	-	0.4	-	-	0.5	-
2393	14-Hydroxy-isocaryophyllene (=β-Betulenol) (**4**)	0.9	1.3	1.2	1.3	1.1	1.0	-	0.6	0.6	0.2	0.1	0.8	0.4	0.5	tr
2400	Tetracosane	-	-	-	-	-	-	0.7	-	-	-	-	-	0.2	-	-
2415	14-Hydroxy-4,5-dihydro-β-caryophyllene (**8**)	13.4	21.4	17.2	14.3	24.8	16.0	19.1	18.5	36.8	4.2	3.7	27.6	22.7	25.2	23.8
2478	14-Hydroxy-α-humulene	1.0	1.4	1.7	1.2	0.4	1.1	0.6	0.7	3.3	0.2	-	4.9	0.5	1.4	3.5
2500	Pentacosane	5.3	1.3	0.5	1.3	2.0	-	13.6	3.6	0.4	0.2	1.2	1.6	13.3	1.2	0.2
2609	14-Hydroxy-4,5-epoxy-β-caryophyllene (*ββ*) (**7**)	-	0.2	0.2	tr	-	0.1	-	-	-	-	-	-	-	0.1	-
2617	14-Acetoxy-4,5-epoxy-β-caryophyllene (*βα*) (**6a**)	-	-	tr	tr	-	-	-	-	-	-	-	-	-	0.2	-
2622	Phytol	0.1	0.2	tr	0.1	0.2	-	0.1	0.6	-	-	1.6	0.1	0.7	0.4	-
2663	14-Hydroxy-4,5-epoxy-β-caryophyllene (*βα*) (**6**)	0.2	0.9	1.3	0.3	0.2	0.4	-	0.3	0.9	-	0.1	0.3	-	0.5	0.7
2700	Heptacosane	3.2	0.2	0.3	0.6	0.8	-	3.0	2.4	-	0.1	1.4	1.7	8.6	1.0	-
2931	Hexadecanoic acid	0.4	0.2	0.1	1.0	0.4	-	0.9	0.5	-	0.1	3.1	0.1	1.0	0.1	-

Br: Branch, L: Leaf, B: Bud
RRI Relative retention indices calculated against *n*-alkanes
% calculated from TIC data
tr Trace (< 0.1 %)

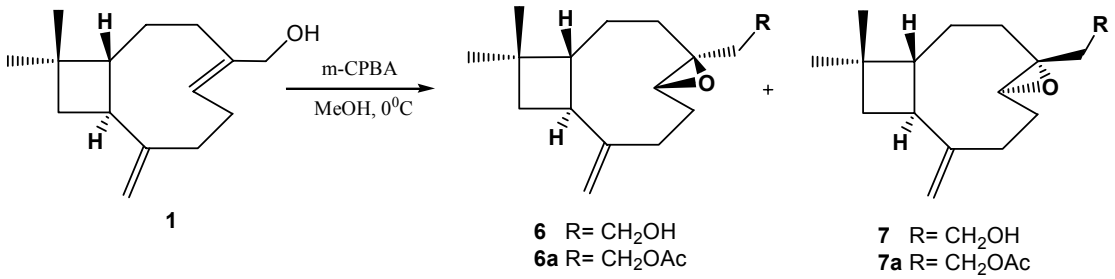
Manns and Hartmann reported (4*E*)-isocaryophyllen-14-al (=β-betulenal) in *Cunila spicata* Benth. (Lamiacea).[26] Barrero *et al*.[27] and Hiede *et al*.[28] reported the presence of betulenal in *Juniperus oxycedrus* and *J. virginiana* essential oils without its configuration. Kaiser and Lamparsky[29] assigned the structure of the aldehyde, formed in the reaction mixture by direct oxidation of caryophyllene with SeO_2, as caryophyllen-14-al (=α-betulenal), which was also detected in lavender oil.

With the same intention as above we acetylated 14-hydroxy-isocaryophyllene (**4**), resulting in the formation of 14-acetoxy-isocaryophyllene (=β-betulenol acetate) (**4a**). However, the acetate **4a** was not detected in any *Betula* essential oils investigated in this study (Scheme 3).



Scheme 3. Acetylation of 14-hydroxy-isocaryophyllene (**4**).

Furthermore, 14-hydroxy-β-caryophyllene (**1**) was epoxidized by *m*-CPBA resulting in the formation of the two synthetic diastereomeric epoxides namely, 14-hydroxy-4,5-epoxy-β-caryophyllene (*βα*) (**6**) and 14-hydroxy-4,5-epoxy-β-caryophyllene (*ββ*) (**7**), as shown in Scheme 4.[16] These compounds were shown to be present in the investigated *Betula* essential oils (Table 1). 14-Hydroxy-4,5-epoxy-β-caryophyllene (*βα*) (**6**) was also obtained from the bud essential oil of *B. pendula* by MPLC. The acetate of this compound (**6a**) was shown to be present in the composition of the investigated *Betula* essential oils. The acetate of *ββ*- form (**7a**) was also found in essential oil of *B. recurvata* leaves in trace amounts (Table 1).

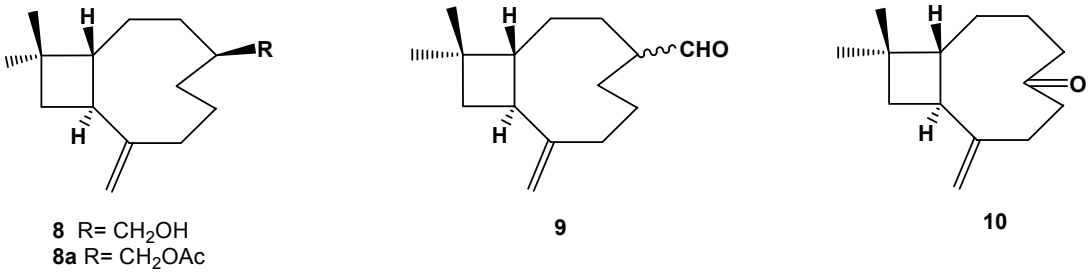


Scheme 4. Epoxidation of 14-hydroxy-β-caryophyllene (**1**).

In vitro antimicrobial activity evaluation against selected human pathogens *Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus cereus* and the fungus *Candida glabrata* using 14-hydroxy-β-caryophyllene (**1**), 14-acetoxy-β-caryophyllene (**1a**), β-betulenal (**2**), β-caryophyllene (**3**), and 14-hydroxy-isocaryophyllene (**4**) were conducted. Chloramphenicol was used as reference and moderate activities were observed against Gram

(+)/(-) bacteria. Ketoconazole was used as antifungal reference against *C. glabrata*, where also moderate activity was observed. Antibacterial activity was shown against *Streptococcus nutans*, *S. aureus* and *E. coli* for caryophylla-4,8(13)-dien-6-ol, caryophylla-4,8(13)-dien-6-one, caryophylla-4,7-dien-6-one and caryophylla-3,8(13)-dien-5,6-diol isolated from *B. pubescens*, activities of which were said to be patented.[30] Other antimicrobial investigations using different extracts of various *Betula* species have been conducted.[31-34] Recently, 14-hydroxy-β-caryophyllene (**1**) was reported to have antibacterial activity against *Bacillus subtilis* and *Escherichia coli*.[24]

Our research into *Betula* species growing in Turkey has resulted in the isolation of new caryophyllene derivatives namely; 14-hydroxy-4,5-dihydro-β-caryophyllene (**8**), 4,5-dihydro-β-caryophyllene-14-al (**9**), and *des*-4-methyl-caryophyll-8(14)-en-5-one (**10**) from *B. litwinowii.*[15] 14-Acetoxy-4,5-dihydro-β-caryophyllene (**8a**) was prepared from 14-hydroxy-4,5-dihydro-β-caryophyllene (**8**) (Figure 1). 14-Acetoxy-4,5-dihydro-β-caryophyllene (**8a**) was also identified by GC-MS and retention index data to be identical to that present in the *Betula* essential oils (Table 1).



8 R= CH_2OH
8a R= CH_2OAc

9

10

Figure 1. Isolated and semi-synthetic new compunds from *B. litwinowii.*

14-hydroxy-4,5-dihydro-β-caryophyllene (**8**) induced 100% inhibition of the plant pathogenic fungi *Cephalosporium aphidicola* and *Rhizoctonia cerealis* at 200 μg/mL. This compound was also as active as the antibacterial standard chloramphenicol against *Bacillus cereus*, with a MIC value of 125 μg/mL, but was less active against *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Same compound displayed moderate antifungal activity against *Candida glabrata*, having a MIC value of 125 μg/mL when compared to ketoconazole (62.5 μg/mL).[15]

The leaves of five *Betula* species growing in Turkey, were hydrodistilled and the oil compositions were investigated by GC-MS. 14-Hydroxy-β-caryophyllene (**1**) was found as the main constituents (29.3%) in the oil of *B. pendula*. 14-Hydroxy-4,5-dihydro-β-caryophyllene (**8**) was identified as the main constituents in the oils of *B. recurvata* (25.%), *B. browicziana* (24.8%) and *B. litwinowii* (18.5%). Interestingly, in the oil of *B. medwediewii,* methyl salicylate (49.8%) was the major constituent.[14]

14-Hydroxy-β-caryophyllene (**1**) was found as main compound in the hydrodistilled branch oil of *B. pendula* and *B. browicziana* (19.8% and 18.0%, respectively). 14-Hydroxy-4,5-dihydro-β-caryophyllene (**8**) was characterized as the main component *B. recurvata* and *B. litwinowii*

branch oils (22.7 and 19.1%, respectively). Methyl salicylate (67.7%) was identified as a major compound in the oil of *B. medwediewii* (Table 1).

Various phytopathogenic fungi were evaluated by agar tube dilution method[35] to test the antifungal activities of the leaf essential oils of *B. pendula, B. browicziana, B. medwediewii, B. recurvata* and *B. litwinowii* at 400 μg/mL concentration. *Cephalosporium aphidicola, Drechslera sorokiniana, Fusarium solani, Rhizoctonia cerealis,* were inhibited, whereas weak activity or no inhibition was observed against *Aspergillus quadrilieneatus, A. flavus, Gibberella fujikuroi, Trichoderma harzianum* and *Trichothecium roseum*.[14] Previous studies demonstrated the antifungal activity of some *Betula* species; *B. alba* [36], *B. lenta*[37], *B. nigra*[38], *B. papyrifera*[39] and *B. plathyphylla* var. *japonica*.[40]

In the course of our research into *Betula* species, we isolated essential oils from the buds of *Betula pubescens* ssp. *pubescens* and *B. pubescens* ssp. *czerepanovii* naturaly growing in Finland which were analyzed both by GC and GC-MS. 14-Acetoxy-β-caryophyllene (**1a**) was determined as the main component in both oils (32.5 and 30.0%, respectively). The essential oil was subjected to column chromatography and a bicyclic aldehyde; birkenal (**11**) and a tricyclic lactone; hushinone (**12**) were isolated as new compounds. Birkenal (**11**) was subjected to a mild reduction with $NaBH_4$ to result in birkenol (**13**). This compound was shown to be naturally present in both essential oils (0.4-0.6%) with the aid of GC-MS (Table 2). The acetate of this alcohol; birkenyl acetate (**13a**) was shown to be naturally present at low concentrations (0.1%) in both essential oils investigated, as a new natural compound. 6-Hydroxycaryophyllene (**14**) was also isolated from the oils. Acetylation of this compound resulted in the formation of 6-acetoxycaryophyllene (**14a**) (Figure 2). The new acetate was also detected in the essential oils and identified as such by co-elution by means of TLC and GC-MS.[17] Recently, Domrachev and Tkachev assigned the absolute configuration of birkenal by chemical correlation with known caryophyllene-type derivatives.[41]

The air-dried buds were hydrodistilled for 3 h using a Clevenger-type apparatus to yield 5.0% (A) and 7.8% (B) of essential oils on a dry-weight basis.

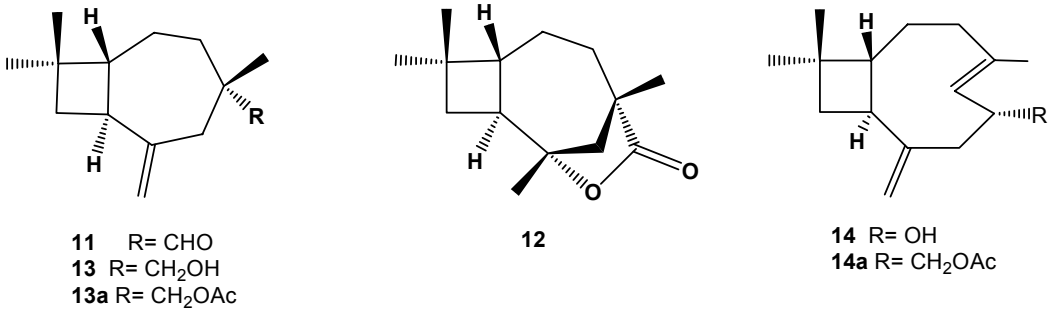
The antioxidant activities of the essential oils from both species and the isolated pure compounds namely; birkenal (**11**), hushinone (**12**), birkenol (**13**), and 6-hydroxycaryophyllene (**14**) were assessed by measuring their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl radicals ($DPPH^{\bullet}$). The test was performed on the samples at concentrations of 0.5 and 1.0 mg/mL but scavenging activity of the radicals was not determined.[17]

Other recent work of our group on volatiles of the buds of *B. pendula* obtained by hydrodistillation and microdistillation collected from Germany was reported. The volatiles were analyzed both by GC and GC-MS systems. α-Copaene (12% and 10%), germacrene D (11% and 18%) and δ-cadinene (11% and 15%) were identified as the main constituents in the hydrodistilled and microdistilled samples, respectively. In this study, the essential oil profile of *B. pendula* obtained from the buds (Table 3) was quite different from those of previous investigations and results.[13,14,16] Kaneko *et al*.[7] had reported δ-cadinene (9.6%) as the main constituent of *B. pendula* from Japan. However, in other previous studies betulenols were found to be the major constituents in the volatile oil of *B. pendula*.[13,14, 16, 42]

Table 2. Essential oil compositions the buds of *B. pubescens* ssp. *pubescens* (A) and *B. pubescens* ssp. *czerepanovii* (B)

RRI	Compound	A (%)	B (%)
1612	β-Caryophyllene (**3**)	0.3	0.7
1823	Birkenal (**11**)	11.7	10.8
2008	Caryophyllene oxide	3.1	3.5
2009	Birkenyl acetate (**13a**)	0.1	0.1
2071	Humulene epoxide II	0.4	0.5
2100	Heneicosane	1.3	0.4
2149	Birkenol (**13**)	0.4	0.6
2193	β-Betulenal (**2**)	1.1	1.7
2209	Hushinone (**12**)	0.7	0.2
2210	6-Acetoxycaryophyllene (**14a**)	5.0	1.0
2272	14-Acetoxy-β-caryophyllene (**1a**)	32.5	30.0
2300	Tricosane	1.7	2.6
2316	Caryophylla-2(12),6(13)-dien-5β-ol (=Caryophylladienol I)	1.2	1.3
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol II)	5.2	5.8
2329	14-Acetoxy-α-humulene	1.2	0.7
2346	6-Hydroxycaryophyllene (**14**)	11.7	15.1
2357	14-Hydroxy-β-caryophyllene (**1**)	1.7	3.5
2617	14-Acetoxy-4,5-epoxy-β-caryophyllene ($\beta\alpha$) (**6a**)	1.3	1.8
2663	14-Hydroxy-4,5-epoxy-β-caryophyllene ($\beta\alpha$) (**6**)	-	0.2

RRI Relative retention indices calculated against *n*-alkanes
%: calculated from FID data
tr: trace (< 0.1 %)



11 R= CHO
13 R= CH_2OH
13a R= CH_2OAc

12

14 R= OH
14a R= CH_2OAc

Figure 2. Isolated and semi-synthetic compounds from *Betula pubescens*.

Conclusions

Betula species display an important resource for sesquiterpenes in particular for caryophyllene derivatives. Their biological activity is also worthwhile to investigate as in *in vitro* pre-screens we have observed antimicrobial activity against various human and plant pathogens. Another interesting aspect of sesquiterpenes and caryophyllenes is the potential use in flavour and fragrance industries, however, the mentioned secondary metabolites need to be investigated further from this aspect.

Experimental Section

Plant material. Leaf, branch and buds of *B. pendula*, *B. browicziana*, *B. litwinowii*, *B. recurvata* and *B. medwediewii* were collected from different localities in North Eastern region of Turkey. Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey (ESSE). Detailed information on the plant materials used are given in Table 4. Buds of *B. pubescens* ssp. *czerepanovii* and *B. pubescens* ssp. *pubescens* were collected in April 2002 from the Botanical Garden of the University of Turku (SW Finland). Voucher specimens of the buds have been deposited in the Turku University Herbarium under numbers TUR 573172 and TUR 573171, respectively. Buds of *B. pendula* growing in Germany, were collected from Maxhütte, Regensburg in April 2002.
The plant materials were either hydrodistilled using a Clevenger type apparatus or were subjected to Likens-Nickerson simultaneous distillation-extraction (SDE) method and Microdistillation method when the plant material amounts were insufficient. The essential oils were analysed by GC and GC-MS.

Isolation of the essential oils
Hydrodistillation. The plant materials were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the essential oils. The percentage (%) yields were calculated on dry weight basis after drying over anhydrous Na_2SO_4.
Likens-Nickerson distillation-extraction method. *B. browicziana* and *B. recurvata* (1.0 g of buds) were subjected to SDE for 1 hour using a Likens-Nickerson apparatus with 1 ml of *n*-hexane as solvent.
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. *n*-Hexane (300 µl) was also added into the collecting vial to trap the volatile components. The sample vial was heated to 100ºC at a rate of 20ºC/min and then kept at 100ºC for 15 min. It was then heated to 112ºC at a rate of 20ºC/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 –5ºC during the distillation. After the distillation was completed the *n*-hexane-trapped volatiles were analyzed by both by GC and GC-MS.

Table 3. Main components of *Betula pendula* buds growing in Germany

RRI	Compound	Hydrodistillation	Microdistillation
1400	Nonanal	0.9	tr
1466	α-Cubebene	0.8	0.5
1493	α-Ylangene	1.1	0.7
1497	α-Copaene	11.8	9.6
1549	β-Cubebene	0.7	0.5
1589	β-Ylangene	1.3	0.4
1597	β-Copaene	1.0	0.5
1612	β-Caryophyllene (**3**)	3.4	3.2
1617	6,9-Guaiadiene	2.4	1.9
1628	Aromadendrene	0.6	0.3
1661	Alloaromadendrene	2.2	2.2
1677	*epi*-Zonarene	0.6	0.6
1687	α-Humulene	2.9	3.0
1704	γ-Muurolene	2.6	3.0
1726	Germacrene D	11.4	18.0
1740	α-Muurolene	2.0	2.5
1773	δ-Cadinene	10.8	15.3
1776	γ-Cadinene	2.4	4.0
1810	3,7-Guaiadiene	0.5	0.7
1941	α-Calacorene	0.7	0.5
2008	Caryophyllene oxide	0.5	0.7
2071	Humulene epoxide-II	0.5	0.6
2080	Cubenol	2.7	0.6
2088	1-epi-Cubenol	5.0	1.4
2109	Furopelargone B	0.9	1.4
2187	T-Cadinol	1.5	3.4
2209	T-Muurolol	0.9	1.7
2219	δ-Cadinol	0.4	0.7
2255	α-Cadinol	2.8	5.8
2300	Tricosane	0.5	0.3
2369	Eudesma-4(15),7-dien-1β-ol	0.1	0.7
2500	Pentacosane	1.6	2.8

RRI: Relative retention indices calculated against *n*-alkanes
%: calculated from FID data
tr: Trace (< 0.1 %)
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%.

Table 4. Information on the *Betula* species growing in Turkey and essential oils

Betula species	Collection Site	Altitude (m)	Collection Period	Parts	Oil Yield* (%)	ESSE
browicziana	Rize - Çamlıhemşin	1765	07.1996	Branch	0.15	12239
	Rize - Çamlıhemşin	1765	07.1996	Leaf	0.11	12239
	Rize - Çamlıhemşin	1765	05.1998	Bud	#	12760
litwinowii	Artvin - Hatila valley	2050	07.1998	Branch	0.01	12755
	Artvin - Hatila valley	2050	07.1998	Leaf	0.17	12755
	Artvin - Hatila valley	2050	05.1998	Bud	6.34	12757
medwediewii	Rize - Çamlıhemşin	1700	05.1998	Branch	0.1	12759
	Rize - Çamlıhemşin	1700	06.1998	Leaf	0.13	12563
	Rize - Çamlıhemşin	1700	05.1998	Bud	1.25	12759
pendula	Erzurum	1800	05.1998	Branch	0.1	12527
	Erzurum	1800	05.1998	Leaf	0.63	12527
	Erzurum	1800	05.1998	Bud	3.82	12527
recurvata	Rize - Çamlıhemşin	1700-1800	06.1998	Branch	**	12534
	Rize - Çamlıhemşin	1700-1800	06.1998	Leaf	0.56	12534
	Rize - Çamlıhemşin	1700	09.1998	Bud	#	12758

*Yields are given on moisture free basis

** Due to the poor yield of oil, it was dissolved in *n*-hexane.

Likens-Nickerson SDE

Analysis of the essential oils

Gas chromatography (GC). *Betula* essential oils were analyzed by GC using a Hewlett Packard 6890 system and an HP Innowax FSC column (60 m x 0.25 mm ∅, with 0.25 μm film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60°C for 10 min, and increased at 4°C/min to 220°C, then constant at 220°C for 10 min and increased at 1°C/min to 240°C. Injector temperature was set at 250°C. Percentage composition of the individual components were obtained from electronic integration using flame ionization detection (FID) at 250°C. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentages of the characterized components were as cited in Table 1-3.

Gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was performed with a Hewlett-Packard GCD, system and Innowax FSC column (60 m x 0.25 mm ∅, 0.25 μm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 425.

Identification of components. Identification of the essential oil components were carried out by comparison of individual relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley and MassFinder 2.1) and in-house "Baser Library of Essential Oil Constituents" libraries made up by genuine compounds and components of known oils, as well as MS literature data was also used for the identification.

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