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THE COMPOSITION AND ANTIFUNGAL BIOASSAY OF THE ESSENTIAL OILS OF DIFFERENT *Betula* SPECIES GROWING IN TURKEY

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Five Betula *species,* B. pendula, B. browicziana, B. medwediewii, B. litwinowii, *and* B. recurvata, *were collected from different parts of Turkey. The leaves of these species were hydrodistilled to yield the consequent essential oils. The essential oil compositions were investigated by GC/MS. 14-Hydroxy-β-caryophyllene was the main constituent in the oil of* B. pendula. *14-Hydroxy-4,5-dihydro-β-caryophyllene, a new compound, was identified as the main constituent in the oils of* B. browicziana, B. litwinowii, and B. recurvata. *In the oil of* B. medwediewii *methyl salicylate was the main compound. Various phytopathogenic fungi were studied by the agar tube dilution technique to test the antifungal activities of the essential oils at 400 μg/ml concentration. The essential oils showed strong antifungal activity against* Cephalosporium aphidicola, Drechslera sorokinianse, Fusarium solani, *and* Rhizoctonia cerealis.

Key words: *Betula* species, essential oil, GC/MS, antifungal activities, *Cephalosporium aphidicola, Drechslera sorokinianse, Fusarium solani, Phizoctonia cerealis.*

Betula species, commonly known as the birch tree and generally characterized by a white bark which peels off like paper have a wide distribution from Canada to Japan and in various parts, altitudes, and climates of the world [1, 2]. The genus *Betula* is represented by 5 species in the Eastern and North-Eastern parts of Turkey [3–5].

Birch trees have a long history of medicinal use in different countries and cultures. *Betula* species are of special interest in cosmetic preparations and medicines due to their biological activities. Dried bark, trunk bark, wood, roots, leaves, buds, resin, fresh sap and leaves, tar and essential oils, and virtually all parts of the tree have been used to treat a wide spectrum of diseases and complications. Various preparations from *Betula* species have been reported which have diuretic, purgative, antiemetic, antipyretic, blood rectifiying, antihypertensive, antiseptic, nematocidal, and insecticidal activities. Also many uses against simple injuries, cuts, wounds, dermatitis, acne, rheumatism, hysteria, bronchitis, cough, diabetes, furunculosis, sciatica, menorrhagia, kidney diseases, convulsive disorders, and atherosclerosis have been reported [2, 5–10].

The antimicrobial effects of plant materials commonly used in food, drug, and cosmetic products have been recognized for a long time. The antimicrobial effects of plant extracts, aromatic chemicals, and especially essential oils have been evaluated against bacteria and molds showing strong activity [11–15].

Several investigators have studied the essential oil composition of various parts of *Betula* species [16–27]. Recently the essential oils and compounds of *Betula* species growing naturally in Turkey were the subject of several studies of our group [28]. This paper illustrates the antifungal activities of *Betula* essential oils obtained from *Betula* species growing in various regions of Turkey against some phytopathogenic fungi. Although the selected fungi were phytopathogenic, some have also been reported to be pathogenic or allergenic to humans in some cases [29, 30].

The essential oils of the leaves of *Betula* species were analyzed by GC/MS. The essential oil of the most popular *Betula* species, *B. pendula*, consisted of 55 compounds representing 86.7% of the total oil, in which 14-hydroxy-β-caryophyllene (=α-betulenol) was identified as the main component (29.3%). Betulenols were previously identified as the main components in many

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TABLE 1. Composition (%) of the Essential Oils of *Betula* Species

Compound	BP	BB	BM	BR	BL
Hexanal	0.1	0.1	0.5	0.1	-
Heptanal	Tr.	0.3	Tr.	0.2	Tr.
(*Z*)-3-Hexanal	0.2	0.4	5.8	0.4	Tr.
1-Hexanol	0.1	0.1	1.4	0.2	0.2
(*Z*)-3-Hexen-1-ol	-	-	0.3	0.1	-
Nonanal	0.1	0.6	1.8	0.2	0.1
(*E*)-2-Hexen-1-ol	-	-	0.7	0.1	0.1
(*E*)-2-Octenal	-	0.3	-	0.1	0.1
trans-Linalool oxide (furanoid)	-	-	-	Tr.	0.1
α-Cubebene	Tr.	-	-	-	Tr.
cis-Linalool oxide (furanoid)	-	-	-	Tr.	0.1
cis-3-Nexenyl-2-methylbutyrate	-	-	-	0.1	Tr
Pentadecane	-	-	-	-	0.2
α-Copaene	Tr.	-	-	Tr.	0.2
(*E,E*)-2,4-Heptadienal	-	-	-	Tr.	0.2
Decanal	-	-	0.2	0.1	0.2
Benzaldehyde	Tr.	-	-	Tr.	-
(*E*)-2-Nonenal	-	-	-	-	0.2
β-Cubebene	Tr.	-	-	0.2	0.2
Linalool	0.1	0.5	2.8	0.2	1.1
Hexadecane	-	-	Tr.	-	0.1
6-Methyl-3,5-heptadien-2-one	-	0.1	-	-	-
β-Caryophyllene	1.4	0.3	0.4	1.3	1.1
Hexyl hexanoate	-	-	0.3	-	-
Hotrienol	-	-	-	-	0.1
Hexyl tiglate	Tr.	-	-	0.1	0.1
p-Menth-1-en-9-al	-	-	-	0.1	0.1
β-Cyclocitral	Tr.	0.1	0.3	-	0.1
(*E*)-2-Decenal	-	0.2	-	-	0.2
Benzene acetaldehyde	0.1	0.2	0.2	0.2	-
(*Z*)-3-Hexenyl tiglate	Tr.	-	-	0.1	0.1
α-Humulene	2.0	0.3	0.2	1.7	0.7
Heptadecane	-	-	-	-	0.1
α-Terpineol	Tr.	0.1	0.5	0.1	0.3
Germacrene-D	Tr.	-	-	Tr.	-
Dodecanal	-	-	0.2	Tr.	Tr.
β-Selinene	0.1	-	-	Tr.	Tr.
α-Selinene	0.1	-	-	-	Tr.
Geranial	-	-	0.3	0.1	Tr.
(*E,E*)-α-Farnesene	-	-	1.9	0.1	0.6
Citronellol	Tr.	0.1	-	0.1	0.7
δ-Cadinene	0.1	-	-	Tr.	-
Octadecane	-	-	-	-	0.1
Methyl salicylate	-	-	**49.8**	-	-
Nerol	-	-	0.2	0.1	0.1
Cumin aldehyde	-	0.8	-	-	-
p-Mentha-1,3-dien-7-al	-	0.1	-	-	-
Ethyl salicylate	-	-	0.2	-	-

TABLE 1. (continued)

Compound	BP	BB	BM	BR	BL
β-Damascenone	Tr.	0.1	-	0.1	0.1
Geraniol	0.1	1.1	1.8	0.6	2.0
(*E*)-Geranylacetone	-	0.4	0.3	Tr.	0.1
α-Calacorene	Tr.	-	-	-	-
trans-Jasmone	-	0.1	-	-	-
Cubebol	0.7	-	-	-	-
β-Ionone	0.6	0.3	1.0	Tr.	0.2
Dihydro-β-ionol	-	-	0.2	Tr.	-
Isocaryophyllene oxide	0.1	-	-	0.1	-
Cacyophyllene oxide	4.3	2.3	0.5	2.9	3.1
Caryophylla-8(14)-en-5-one*	4.7	10.2	0.8	6.9	5.7
Salvial-4(14)-en-1-one	Tr.	-	-	-	-
Humulene epoxide-I	0.3	0.2	-	0.2	0.1
Pentadecanal	-	-	0.2	-	-
(*E*)-Nerolidol	-	-	0.2	-	0.1
Humulene epoxide-II	4.8	2.4	0.4	2.6	1.5
Caryophylla-4(14),8(15)-dien-5-one	-	0.2	-	-	0.6
Humulene epoxide-III	0.1	-	-	0.1	0.1
4,5-Dihydro-β-caryophyllene-14-al*	2.3	0.4	0.1	2.2	0.6
Hexylbenzoate	-	-	0.1	-	-
Heneicosane	0.1	-	-	-	0.3
Hexahydrofarnesylacetone	-	0.2	-	Tr.	0.1
Nonanoic acid	-	-	0.3	-	-
Hexadecanal	-	-	-	-	0.3
(*Z*)-3-Hexene-1-yl benzoate	-	Tr.	-	Tr.	0.3
Nonanoic acid	-	-	-	0.1	-
Eugenol	0.2	0.2	0.9	0.6	-
β-Betulenal	4.7	13.9	0.8	5.2	7.3
T-Muurolol	Tr.	-	-	-	-
Phenylethyl tiglate	0.1	-	-	-	-
Docosane	-	-	-	-	1.1
Carvacrol	Tr.	2.4	0.6	0.1	0.1
α-Cadinol	0.2	-	-	-	-
14-Acetoxy-β-caryophyllene	0.6	0.5	-	2.3	1.0
14-Acetoxy-4,5-dihydro-β-caryophyllene*	0.2	0.2	-	0.3	0.4
Decanoic acid	-	-	0.2	-	-
Tricosane	-	-	0.1	-	1.1
Caryophylladienol-I(=*Caryophylla-4(14),8(15)-dien-5β-ol*)	0.6	0.8	0.4	0.7	0.7
Caryophylladienol-II(=*Caryophylla-4(14),8(15)-dien-5α-ol*)	2.1	1.8	-	2.1	2.6
14-Acetoxy-α-humulene	0.2	-	-	0.1	0.2
Caryophyllenol-I(=*Caryophylla-4(14),6-dien-5α-ol*)	-	0.2	-	-	-
14-Hydroxy-β-caryophyllene(=*α-Betulenol*)	**29.3**	12.7	3.5	20.8	13.2
Caryophyllenol-II(=*Caryophylla-4(14),6-dien-5β-ol*)	-	-	-	0.4	-
14-Hydroxy-isocaryophyllene(=*β-Betulenol*)	1.3	1.1	0.1	0.5	0.6
Hexadecanol	-	1.3	0.4	0.5	0.3
14-Hydroxy-4,5-dihydro-β-caryophyllene*	21.4	**24.8**	3.7	**25.2**	**18.5**
14-Hydroxy-α-humulene	1.4	0.4	-	1.4	0.7
Dodecanoic acid	-	-	0.3	-	0.1

TABLE 1. (continued)

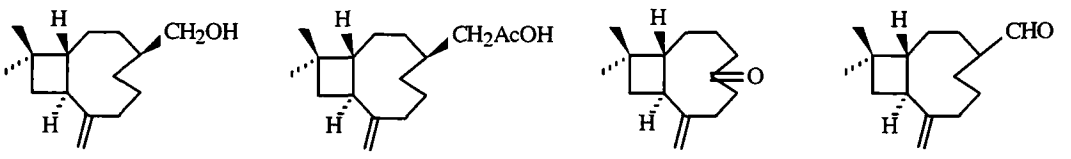
Compound	BP	BB	BM	BR	BL
Pentacosane	1.3	2.0	1.2	1.2	3.6
Ethyl linoleate	-	-	-	-	0.1
Geranyl linalool	-	-	0.4	-	-
Methyl linolenate	-	-	-	-	0.2
Hexacosane	-	-	-	-	0.1
Ethyl linolenate	-	-	-	-	0.1
Phytol	0.2	0.2	1.6	0.4	0.6
Heptacosane	0.2	0.8	1.4	1.0	2.4
Tetradecanoic acid	-	-	0.4	-	0.1
Nonacosane	-	-	-	-	0.1
Hexadecanoic acid	0.2	0.4	3.1	0.1	0.5
Total	**86.7**	**86.2**	**93.0**	**84.7**	**78.4**

Tr.: trace.

*New compounds [30].

TABLE 2. Growth Inhibition of Some Fungi by *Betula* ssp. Essential Oils

Fungi	BP	BB	BM	BR	BL
Cephalosporium aphidicola	100	100	100	100	100
Trichoderma harzianum	50	50	0	62.5	50
Drechslera sorokinianse	87.5	65	50	87.5	87.5
Aspergillus quadrilineatus	12.5	12.5	0	50	25
Aspergillus flavus	25	37.5	0	50	25
Fusarium solani	50	37.5	12.5	87.5	56
Rhizoctonia cerealis	100	100	100	100	100
Gibberella fujikuroi	12.5	0	0	-	12.5
Trichothecium roseum	50	25	12.5	44	37.5



1 2 3 4

Fig. 1

TABLE 3. Information of *Betula Essential* Oils

Betula species	Code	ESSE[1]	Collection Place	Altitude	Collection	Yield[2] (%)
B. pendula	BP	12527	Erzurum	1800 m	May, 1998	0.63
B. browicziana (E)[3]	BB	12239	Rise: Camlihemsin	1765 m	July, 1996	0.11
B. medwediewii	BM	12563	Rise: Camlihemsin	1700 m	June, 1998	0.13
B. recurvata	BR	12534	Rise: Camlihemsin	1800 m	June, 1998	0.56
B. litwinowii	BL	12755	Artvin	2050 m	July, 1998	0.17

[1] Herbarium of the Faculty of Pharmacy at Anadolu University in Eskisehir.

[2] Yields are given on moisture dry basis.

[3] (E) Endemic.

Betula species [17, 19–21, 27]. *Betula pendula* was mainly investigated for its bud oil by Russian investigators from Yakutia, and α-betulenol acetate (21.9%), caryophyllene (23.2%), humulene (5.8%), caryophyllene oxide (6.5%), and α- and β-betulenols (4.7 and 6.5%, respectively) were reported as the main components [24, 26].

A total of 47, 65, and 77 compounds were identified from *B. browicziana*, *B. recurvata*, and *B. litwinowii* leaf oils representing 86.2, 84.7, and 78.4% of the total essential oils, respectively. Interestingly, in all three species, the new compound 14-hydroxy-4,5-dihydro-β-caryophyllene (**1**) was characterized as the main component, as shown in Table 1. 14-Acetoxy-4,5-dihydro-β-caryophyllene (**2**), caryophylla-8(14)-en-5-one (**3**), and 4,5-dihydro-β-caryopyllene-14-al (**4**) were also previously elucidated as new compounds (Fig. 1) in *Betula* oils growing in Turkey [28].

Analysis of *B. medwediewii* oil resulted in the characterization of 51 compounds representing 93% of the total essential oils, in which methyl salicylate (49.8%) was the main component, as previously reported in other *Betula* species [18, 25, 27].

Table 2 shows the effects of five different essential oils on the growth of nine phytopathogenic fungi. The essential oils of *B. pendula*, *B. browicziana*, *B. medwediewii*, *B. recurvata*, and *B. litwinowii* at 400 μg/ml concentration completely inhibited the growth of *Cephalosporium aphidicola* and *Rhizoctonia cerealis*. Therefore, these two fungi were found to be the most sensitive organisms. However, growth inhibition of *Drechslera sorokinianse* and *Fusarium solani* was somewhat weaker. Other fungi showed differing degrees of growth inhibition.

The essential oil obtained from the leaves of *B. recurvata* showed the highest antifungal activity against all fungi tested except *Gibberella fujikuroi*. Unfortunately, *Gibberella fujikuroi* could not be tested with *B. recurvata* essential oils. This fungus seems to be the most resistant fungus against *Betula* essential oils. *Trichoderma harzianum* was moderately inhibited by *Betula* essential oils, and no inhibition was observed with *B. medwediewii* essential oils. Both *Aspergillus quadrilineatus* and *Aspergillus flavus* were very weakly affected by the oils, and moderately inhibited by the essential oils obtained from *B. recurvata*. *Trichothecium roseum* was also inhibited moderately to weakly by the essential oils. *B. pendula* was moderately effective here (50% inhibition), followed in descending order by *B. recurvata*, *B. litwinowii*, *B. browicziana*, and *B. medwediewii* with12.5% inhibition.

Earlier studies demonstrated the antifungal activity of some *Betula* species, namely *B. alba* [33, 34], *B. lenta* [35], *B. nigra* [36], *B. papyrifera* [39], and *B. plathyphylla var. japonica* [38]. Antibacterial evaluations of *Betula* oils have also been reported [22, 23]. As far as our knowledge and literature search show, there has been no previous work on the *Betula* sp. used in our study, especially on the most commonly known species *B. pendula*. This study shows that essential oils from *B. pendula*, *B. browicziana*, *B. medwediewii*, *B. recurvata* and *B. litwinowii* are quite promising for their antifungal action against plant pathogenic fungi.

In conclusion, since the growth inhibitory effects of essential oil components are variable, depending upon the chemical structure of the components and the strains of the organisms [39], more screenings should be performed on various other fungi. Also, it may be worthwhile to investigate the antimicrobial activities of the main components of essential oils.

EXPERIMENTAL

The oil was analyzed by GC/MS using a Hewlett Packard GCD system. An Innowax FSC column (60 m × 0.25 mm) was used with Helium as a carrier gas (1 ml/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, then kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted at 50:1. The injector temperature was at 250°C. MS were recorded at 70 eV. Mass range was from *m/z* 35 to 425. A library search was carried out using the Wiley GC/MS Library and the TBAM Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by computer. The identified compounds are listed in Table 1.

Plant materials were collected from different regions of Turkey by the authors. Voucher specimens are deposited at the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskisehir (ESSE). Leaves were subjected to hydrodistillation using a Clevenger type apparatus for 4 h. Oil yields and information on collection sites are given in Table 3.

Antifungal activity was determined using the agar tube dilution technique [33, 34]. Stock solutions of the essential oils were freshly prepared in dimethylsulfoxide (DMSO) to reach a final concentration of 400 µg/ml using sterile molten Sabouraud dextrose agar (SDA). Test tubes were kept at room temperature for solidification. Medium containing only DMSO was used as negative control. Fungi were cut to 4×4 mm from one-week-old cultures and then inoculated onto the slant. After an incubation period of 7–10 days at 29°C, tubes were examined for growth inhibition. Ketoconazole, which inhibited all fungi 100%, was used as reference antifungal drug. Growth on the media-containing compound was determined by measuring the linear growth (mm) of the fungal culture. Growth inhibition (%) was calculated with reference to the negative control (Table 2). The pathogenic fungi used in this study were obtained from Anadolu University, Faculty of Sciences and HEJ Research Institute of Chemistry , Karachi, Pakistan.

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