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Bioactivities and Compositions of *Betula nigra* Essential Oils

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ABSTRACT

The essential oils of *Betula nigra* (river birch, Betulaceae) buds, leaves, and inner bark were extracted by hydrodistillation and analyzed by GC-MS. The bud essential oils were dominated by eugenol and paraffin hydrocarbons, the leaf oils were rich in (2*E*)-hexenal, linalool, and eugenol, and the bark essential oils were composed largely of fatty acids, paraffin hydrocarbons, and benzenoid aromatics. A screening of the oils for biological activity, including phytotoxic activity against *Lactuca sativa* (lettuce) and *Lolium perenne* (perennial ryegrass), nematocical activity against *Caenorhabditis elegans*, brine shrimp lethality against *Artemia salina*, insecticidal activity using *Solenopsis invicta* × *richteri* (red imported fire ant), and antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. The leaf oil demonstrated notable biological activity in all bioassays.

INTRODUCTION

Birch preparations, such as teas and infusions, have been important as traditional medicines in several cultures worldwide. The oil from *B. alba* bark (birch tar) has been used to preserve leather in northern Europe and has demonstrated insect repellent activity, while a tea prepared from *B. alba* leaves has been used for treatment of gout, rheumatism, and dropsy (Grieve, 1971). In Ayurveda, the essential oil of *B. alba* is used in treating eczema and psoriasis and to combat hair loss (Vinod *et al.*, 2012). A decoction of the inner bark of *B. occidentalis* was used by western Native Americans to treat colds, coughs, and other pulmonary ailments (Lewis and Elvin-Lewis, 1977).

The Makandwewininiwag band of the Ojibwe people used a tea prepared from *B. pumila* buds as a postparturition tonic, while *B. pumila* buds were heated to make an incense to treat respiratory disorders (Lewis and Elvin-Lewis, 1977). Teas made from the bark of *B. lenta* were used by Native Americans to treat stomachaches and lung ailments, and teas made from bark and from twigs were used for treating fevers. The essential oil from *B. lenta* has been used for treatment of rheumatism, gout, scrofula, bladder infections, and neuralgia, and also as an anti-inflammatory analgesic (Foster and Duke, 1990).

Betula nigra L., river birch, is a tree native to the southeastern United States (Grelen, 1990). This woody plant, which has reddish-brown shredding bark and simple, alternate, double-toothed leaves, and which can grow over 20 m in height, was used by Southeastern Native Americans in traditional medicines. The Catawba people boiled *B. nigra* buds to make syrup that was mixed with sulfur for treatment of ringworm and sores (Speck, 1944). The Cherokee chewed leaves to treat dysentery and used a tea made from the bark to treat colds, stomachaches, and urination difficulties (Casey and Wynia, 2010). Creek Indians used *B. nigra* to treat tuberculosis (Hutton, 2010). European-Americans that settled in the Ozark and Ouachita Mountains discovered *B. nigra* was useful in treating wounds and urinary pains (Nolan, 1998).

In this study, the chemical composition of the essential oils extracted from the twigs and buds, the leaves, and the inner bark of *B. nigra* growing in Huntsville, Alabama. To our knowledge, the essential oil compositions of *B. nigra* tissues have not been previously investigated. In addition, the essential oils of *B. nigra* were examined for phytotoxic, nematocidal, insecticidal, and antimicrobial activity.

MATERIALS AND METHODS

Plant material. *Betula nigra* L. (Betulaceae), commonly known as river birch, black birch, and water birch, was used in this study. Plant tissues were collected from the lower branches of five mature individual trees (cultivated transplants, more than 30 years old) growing on the campus of the University of Alabama in Huntsville (34°43′19″ N, 86°38′17″ W, 199 m asl) in full sun. Twig tips and buds were collected February 25 and March 10, 2012; leaves were collected June 2-3, 2012; and inner bark was collected July 3-4, 2012. Identification of the plant material was confirmed by Professor Robert O. Lawton (Department of Biological Sciences, University of Alabama in Huntsville), and a voucher specimen has been placed in the Herbarium of the University of Alabama in Huntsville (voucher number BENI-001).

After collection, the fresh plant materials were stored in sealed plastic bags in a refrigerator until prepared for extraction of the essential oils. For oil extraction, the plant tissues were chopped into small pieces using a knife and then hydrodistilled using a Likens-Nickerson apparatus. The collected essential oils were clear and colorless (Table 1).

Experimental. The essential oils of *B. nigra* were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200°C and interface temperature was 280°C. The GC oven temperature was programed for 40°C initial temperature, hold for 10 min; increase at 3°C/min to 200°C; increase 2°C/min to 220°C. A 1% w/v solution of each essential oil sample in dichloromethane was prepared and 1 µL was injected using a 10:1 split ratio.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080]. The percentages of each component are reported as raw percentages based on total ion current without standardization.

Allelopathic activity. An allelopathic bioassay, based on lettuce (*Lactuca sativa*) and perennial rye grass (*Lolium perenne*) germination and subsequent radicle and hypocotyl growth was measured to study the effects of the *B. nigra* essential oils and components. Stock solutions of each essential oil (4.0 g/L essential oil and 1.0 g/L Tween-80 in water) were prepared and used for the assays. Two-fold serial dilutions of stock test solutions were prepared to give test concentrations of 4000, 2000, 1000, 500, and 250 µg/mL with the control being 1.0 g/L aqueous Tween-80. Seeds were placed in 6-well test plates (10 seeds per well) each well lined with two layers of Whatman No. 1 filter paper moistened with test solution and the test plates were sealed with Parafilm®. The test plates were incubated at room temperature in the dark for 5 days, after which the number of germinated seeds was determined and the root (radicle) and shoot (hypocotyl) lengths were measured.

Antimicrobial screening. The essential oils were screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853), and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique (Satyal, et al., 2012). Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of crude extracts in DMSO plus 50 µL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony-forming units (CFU)/ mL were added to each well

and the plates were incubated at 37°C for 24 h. The final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin was used as a positive antibiotic control and DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* (ATCC No. 10231) in a yeast-nitrogen base growth medium with approximately 7.5×10^7 CFU/mL. Amphotericin B was the positive control. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

Fire ant insecticidal assay. Worker, red imported, fire ants, probably *Solenopsis invicta* × *richteri* hybrid (Chen *et al.*, 2012) were collected from the University of Alabama in Huntsville. Sample solutions of crude extract at 1000 µg/mL, 500 µg/mL and 250 µg/mL were prepared in 1% aqueous Tween-80® solution. The control was 1% Tween solution. Each bioassay was done in triplicate at room temperature using a 40-mL vial, fitted with a filter paper disk on the bottom. The filter paper was sprayed with 600 µL of sample solution and 10 fire ant workers were transferred into each. The mortality of fire ants was recorded after 24 h. LC_{50} values were calculated using the method of Reed-Muench (Reed and Muench, 1938).

Nematocidal assay. A nematocidal assay using *Caenorhabditis elegans* was done using a modification of the procedure of Park et al. (2007). Stock solutions of each essential oil (2.0 g/L essential oil and 1.0 g/L Tween-80 in water) were prepared and used for the assays. Dilutions of the sample solution were made in sterile water solution beginning with 50 µL of the 1% essential oil solution plus 50 µL of sterile water. The sample solution was serially diluted (1:1) with sterile water in a 96-well plate. Into each well, 10-30 *C. elegans* (mixtures of juvenile and adult nematodes, male:female:juvenile ~1:1:2) per 50 µL of sample solution. Sterile water and serially diluted DMSO were used as controls. After 24 h, both the dead and living nematodes were counted using a microscope. Dead nematodes were identified by their immobility and straight body, even after transfer to clean water. LC_{50} values were determined using the Reed-Muench method (Reed and Muench, 1938).

Brine shrimp lethality assay. A brine shrimp (*Artemia salina*) lethality test was done using a modification of the procedure by McLaughlin (1990). *Artemia salina* eggs were hatched in a sea salt solution (Instant Ocean®, 38 g/L) with an incandescent light bulb as the heat source. After 48 hours, the newly hatched nauplii were counted using a micropipette, and 10 nauplii were transferred to each of nine, 20-mL test-vials containing 10 mL of sea salt solution (same as the hatching solution). Of the test vials, three were labeled as controls with one vial containing no DMSO, a second vial containing 10 µL of DMSO, and the third vial containing 100 µL DMSO. The second set of three vials contained 10 µL of 1% essential oil solution in DMSO, and the third set of three vials were prepared by adding 100 µL of 1% essential oil solution in DMSO. Surviving *A. salina* in each test-vial were counted after 24 h. LC_{50} values were determined using the Reed-Muench method (Reed and Muench, 1938).

Result and Discussion

Essential oils were isolated from all three types of collected tissue, although the levels were relatively low, averaging 0.013% for the twigs and buds, 0.034% for the leaves and 0.016% for the inner bark samples (Table 1). The essential oils isolated from the twig tips and buds of *B. nigra* were very complex with a total of 114 compounds identified (Table 2). These oils were dominated by eugenol (28.7-55.7%) and saturated normal alkanes (17.9-44.9%).

Table 1. *Betula nigra* samples and essential oil yields.

Plant tissue	Sample variable	Experimental tree				
		1	2	3	4	5
Twigs & buds	Collection date	2-25-12	2-25-12	2-25-12	3-10-12	3-10-25
	Tissue mass (g)	118.56	119.45	131.69	125.08	117.24
	Essential oil (mg)	23.5	14.7	18.5	17.7	6.6
Leaves	Collection date	6-2-12	6-2-12	6-2-12	6-3-12	6-2-12
	Tissue mass (g)	101.28	64.87	78.30	78.36	68.25
	Essential oil (mg)	20.2	22.5	57.9	53.9	30.9
Inner bark	Collection date	7-3-12	7-3-12	7-3-12	7-4-12	7-3-12
	Tissue mass (g)	93.97	64.78	60.72	59.37	81.33
	Essential oil (mg)	8.5	12.9	11.6	8.9	11.0

Eugenol, in small amounts (≤ 0.3%) has been reported in the essential oils of buds from *Betula* spp. from Turkey (Demirci and Başer, 2003; Başer and Demirci, 2007) and from Russia (Isidorov *et al*., 2004), but was apparently not detected in bud essential oils from *B. pendula* from Germany (Demirci *et al*., 2004) or Estonia (Orav *et al*., 2011) or *B. pubescens* from Finland (Klika *et al*., 2004). These Eurasian *Betula* bud oils were, however, generally dominated by various oxygenated caryophyllene and humulene derivatives.

In contrast, the leaf oils were less complex (30 components) with (2*E*)-hexenal (39.6-57.3%), linalool (9.8-19.2%), and eugenol (6.7-13.5%) as the major components (Table 3). The 45 compounds were identified in the bark essential oil were dominated by fatty acids and fatty-acid-derived compounds (51.2-80.4%) as well as saturated normal alkanes (4.5-29.8%) (Table 4).

Table 2. Composition of twig tip and bud essential oil.

RI[1]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
0801	Hexanal	---	---	---	0.3	0.6
0836	2-Furaldehyde	0.3	0.3	0.2	0.1	0.2
0857	(2*E*)-Hexenal	0.4	0.2	---	0.1	0.5
0857	(3*Z*)-Hexenol	---	---	---	0.3	1.5
0868	(2*E*)-Hexenol	---	---	---	---	0.2
0870	*n*-Hexanol	---	---	---	0.1	0.4
0905	Heptanal	0.4	0.4	tr[2]	0.2	0.1
0960	(3*E*)-Heptenal	---	---	---	0.3	0.1
0962	(3*Z*)-Heptenol	1.0	0.8	---	---	---
0964	Benzaldehyde	---	---	tr	tr	0.1
0973	*n*-Heptanol	---	---	---	tr	---
0981	Hexanoic acid	---	---	0.2	---	0.4
0989	6-Methyl-5-hepten-2-one	---	---	---	tr	0.1
0993	2-Pentylfuran	---	---	---	tr	0.1
1003	2-Ethylbutanoic acid	---	---	---	---	1.0
1005	Octanal	0.2	0.4	tr	---	---
1017	α-Terpinene	---	---	0.3	---	---
1025	*p*-Cymene	---	---	0.3	---	---
1034	**Benzyl alcohol**[3]	2.8	**5.0**	2.4	2.6	2.9
1043	Phenylacetaldehyde	---	---	0.5	0.4	0.9
1056	*o*-Cresol	---	---	---	0.1	0.1
1058	(2*E*)-Octenal	---	---	---	0.2	0.1
1072	*cis*-Linalool oxide (furanoid)	---	---	0.5	0.4	0.2
1089	*o*-Guaiacol	---	---	0.1	0.1	0.1
1100	Linalool	---	---	0.3	---	0.4
1106	**Nonanal**	**6.6**	**4.2**	0.7	3.0	1.9
1114	2-Phenylethyl alcohol	---	---	1.3	0.2	0.3

Table 2. Composition of twig tip and bud essential oil (continued).

RI[a]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
1160	(2*E*)-Nonenal	0.2	0.4	0.1	0.2	tr
1170	*cis*-Linalool oxide (pyranoid)	0.2	0.1	0.2	0.1	0.1
1174	*trans*-Linalool oxide (pyranoid)	0.3	0.4	tr	tr	tr
1180	Octanoic acid	0.6	1.1	0.6	0.4	0.3
1190	α-Terpineol	---	---	0.1	---	0.1
1193	Methyl salicylate	0.5	1.1	0.7	0.4	0.7
1206	Decanal	0.5	0.6	0.1	0.3	0.2
1262	(2*E*)-Decenal	1.8	1.5	0.5	0.8	0.3
1276	Nonanoic acid	1.1	2.1	0.5	0.9	0.7
1294	(2*E*,4*Z*)-Decadienal	0.4	0.3	---	0.3	0.1
1300	Tridecane	0.1	0.1	---	tr	tr
1304	Undecanal	0.2	0.2	tr	tr	tr
1313	*p*-Vinylguaiacol	0.5	0.8	tr	0.4	0.1
1315	(2*E*,4*E*)-Decadienal	0.9	0.5	tr	0.4	0.1
1349	α-Cubebene	tr	tr	---	tr	tr
1360	**Eugenol**	**44.8**	**39.5**	**41.9**	**55.7**	**28.7**
1370	Decanoic acid	0.5	0.5	tr	tr	tr
1377	α-Copaene	tr	0.6	---	tr	tr
1386	Geranyl acetate	tr	tr	---	tr	tr
1393	1-Tetradecene	tr	tr	---	tr	tr
1399	Vanillin	tr	tr	tr	tr	tr
1400	Tetradecane	tr	tr	tr	tr	tr
1410	Dodecanal	tr	tr	tr	tr	---
1420	(*E*)-Caryophyllene	tr	0.4	---	tr	tr
1430	β-Copaene	tr	tr	---	tr	tr
1454	α-Humulene	---	---	---	---	0.4
1479	γ-Muurolene	0.8	1.0	tr	0.5	0.6
1482	Germacrene D	tr	0.4	tr	0.4	3.3
1487	β-Selinene	tr	tr	---	tr	tr
1496	γ-Amorphene	tr	tr	---	tr	tr
1500	Pentadecane	---	---	tr	---	---
1501	α-Muurolene	0.5	0.8	---	tr	0.3
1510	Tridecanal	---	---	---	tr	tr
1514	γ-Cadinene	tr	0.3	---	tr	0.3
1523	δ-Cadinene	tr	0.6	tr	0.4	0.7
1532	*trans*-Cadina-1,4-diene	tr	tr	---	tr	---
1537	α-Cadinene	tr	tr	---	tr	tr
1542	α-Calacorene	tr	tr	---	tr	tr
1562	Dodecanoic acid	tr	tr	tr	tr	tr
1576	Spathulenol	0.5	0.5	---	tr	0.4
1580	Caryophyllene oxide	tr	tr	tr	tr	tr
1590	1-Hexadecene	tr	tr	tr	tr	---
1591	Salvial-4(14)-en-1-one	tr	tr	---	tr	tr
1600	Hexadecane	tr	tr	tr	tr	---
1610	β-Oplopenone + Tetradecanal	1.9	1.2	tr	1.0	0.6
1625	Helifolen-12-al[4]	0.5	tr	tr	tr	tr

Table 2. Composition of twig tip and bud essential oil (continued).

RI[1]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
1629	10-*epi*-γ-Eudesmol	---	---	0.8	---	---
1640	τ-Muurolol	0.7	0.7	---	0.4	0.7
1644	α-Muurolol (= Torreyol)	0.4	tr	---	tr	tr
1646	β-Eudesmol	---	---	1.0	---	---
1650	α-Eudesmol	---	---	1.1	---	---
1652	α-Cadinol	0.8	0.7	---	0.4	0.8
1686	Germacra-4(15),5,10(14)-trien-1α-ol	0.6	tr	---	tr	tr
1691	1-Heptadecene	tr	0.6	---	tr	---
1700	Heptadecane	0.5	1.0	tr	tr	tr
1716	Pentadecanal	tr	0.4	tr	tr	tr
1763	Tetradecanoic acid	0.4	0.7	tr	tr	0.5
1792	1-Octadecene	tr	0.5	tr	tr	---
1800	Octadecane	tr	0.7	tr	tr	---
1815	Hexadecanal	7.9	0.6	tr	0.4	tr
1843	2-Pentadecanone	0.5	0.6	tr	0.4	tr
1875	*n*-Hexadecanol	tr	tr	---	tr	---
1891	1-Nonadecene	tr	tr	tr	tr	tr
1895	Unidentified	---	4.6	---	---	---
1900	Nonadecane	0.6	1.6	tr	tr	tr
1916	Heptadecanal	0.4	tr	tr	tr	---
1916	(*Z*,*Z*)-Geranyl linalool	---	---	---	---	1.3
1952	Unidentified	0.8	0.8	tr	tr	3.6
1960	Palmitic acid	tr	1.6	0.6	0.4	1.4
1966	Unidentified	0.7	---	tr	0.6	**12.6**
1982	(*E*,*Z*)-Geranyl linalool	tr	---	tr	tr	3.5
1991	1-Eicosene	tr	0.8	tr	tr	---
2000	Eicosane	0.6	0.8	tr	tr	---
1997	(*Z*,*E*)-Geranyl linalool	---	---	---	---	4.1
2017	Octadecanal	0.7	0.6	tr	0.6	tr
2090	1-Heneicosene	---	---	tr	---	---
2100	Heneicosane	2.2	2.6	0.9	1.3	0.9
2191	1-Docosene	tr	---	tr	tr	tr
2200	Docosane	0.5	0.5	tr	tr	tr
2271	1-Eicosanol	tr	tr	---	tr	tr
2292	1-Tricosene	---	---	---	tr	tr
2300	**Tricosane**	**5.1**	2.5	1.6	**8.0**	**5.2**
2394	1-Tetracosene	tr	tr	tr	tr	tr
2400	Tetracosane	tr	tr	tr	tr	tr
2495	1-Pentacosene	tr	tr	tr	tr	---
2500	**Pentacosane**	2.1	1.3	1.9	**8.8**	3.6
2595	1-Hexacosene	tr	tr	tr	tr	tr
2600	Hexacosane	tr	tr	tr	tr	tr
2700	**Heptacosane**	**6.2**	**9.0**	**39.1**	**7.6**	**10.5**
2900	Nonacosane	tr	tr	1.4	tr	tr
	Benzenoid aromatics	48.5	46.4	46.9	60.0	33.9
	Paraffin hydrocarbons	17.9	20.2	44.9	25.8	20.2
	Monoterpene hydrocarbons	---	---	0.6	---	---
	Oxygenated monoterpenoids	0.4	0.5	1.1	0.5	0.7
	Sesquiterpene hydrocarbons	1.3	3.9	tr	1.3	5.6
	Oxygenated sesquiterpenoids	4.4	2.5	3.0	1.4	11.1
	Fatty-acid-derived compounds	25.5	20.3	3.2	10.1	11.0
	Others/Unidentified	1.8	5.7	0.2	0.7	16.5
	Total Identified (114)	98.5	94.1	100	99.0	82.7

[1]RI = Retention Index determined with respect to a series of normal alkanes on an HP-5ms column.
[2]tr = trace (< 0.05%).
[3]Abundant components indicated in **boldface**.
[4]Correct isomer not identified.

Table 3. Composition of leaf essential oil.

RI[1]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
0993	6-Methyl-5-hepten-2-one	tr	---	0.4	---	0.7
1008	(3*Z*)-Hexenyl acetate	---	---	0.3	tr	tr
1017	(2*E*)-Hexenyl acetate	---	---	0.5	tr	tr
1033	Benzyl alcohol	1.8	2.2	2.2	1.6	2.2
1072	*cis*-Linalool oxide (furanoid)	tr	tr	1.0	tr	tr
1088	*trans*-Linalool oxide (furanoid)	tr	---	0.6	tr	tr
1100	**Linalool**	**11.4**	**9.8**	**19.2**	**11.6**	**10.0**
1105	Nonanal	2.7	2.6	3.3	3.3	3.5
1169	*cis*-Linalool oxide (pyranoid)	---	---	tr	tr	---
1174	*trans*-Linalool oxide (pyranoid)	---	---	tr	tr	---
1176	Terpinen-4-ol	---	---	tr	---	---
1190	α-Terpineol	2.3	2.0	4.5	2.4	2.0
1193	Methyl salicylate	---	---	tr	---	---
1206	Decanal	---	---	tr	---	---
1227	Nerol	---	---	0.5	tr	---
1252	Geraniol	1.1	0.8	3.5	0.9	0.8
1312	*p*-Vinylguaiacol	1.8	2.5	1.3	1.7	2.2
1357	**Eugenol**	**13.5**	**9.5**	**13.4**	**9.9**	**6.7**
1384	Geranyl acetate	tr	---	---	---	---
1410	Dodecanal	---	---	tr	---	---
2700	Heptacosane	tr	0.9	1.2	0.6	0.5
	Benzenoid aromatics	17.1	14.1	16.9	13.1	11.2
	Paraffin hydrocarbons	tr	0.9	1.2	0.6	0.5
	Monoterpene hydrocarbons	---	---	---	---	---
	Oxygenated monoterpenoids	14.8	12.6	29.3	14.9	12.8
	Sesquiterpene hydrocarbons	---	---	---	---	---
	Oxygenated sesquiterpenoids	---	---	---	---	---
	Fatty-acid-derived compounds	68.1	72.4	52.6	71.4	75.5
	Total Identified (30)	100	100	100	100	100

[1]RI = Retention Index determined with respect to a series of normal alkanes on an HP-5ms column.
[2]tr = trace (< 0.05%).
[3]Abundant components indicated in **boldface**

Leaf oil compositions of *B. pendula*, *B. pubescens*, *B. humilis*, and *B. nana* from Estonia have been reported (Orav *et al.*, 2011). The leaf oils from *B. nigra* in this current study are remarkably dissimilar from the Estonian leaf oils. *B. nigra* leaf oils were dominated by (2*E*)-hexenal, linalool, and eugenol. Neither (2*E*)-hexenal nor eugenol was reported in any of the Estonian *Betula* leaf oil samples, and linalool was only a minor component (≤ 0.8%) in these oils. Additionally, *B. nigra* leaf oils were devoid of oxygenated caryophyllene derivatives such as α-betulenol, β-betulenal, or α-betulenol acetate, which are abundant components found in *B. pendula*, *B. pubescens*, and *B. humilis* leaf oils (Orav *et al.*, 2011).

Table 4. Composition of bark essential oil.

RI[1]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
0801	**Hexanal**[2]	0.9	3.2	**5.8**	0.8	2.8
0835	2-Furaldehyde	0.9	0.7	tr[c]	0.7	1.5
0855	(2*E*)-Hexenal	---	2.0	3.7	---	0.4
0857	**(3*Z*)-Hexenol**	0.4	**6.2**	**7.8**	---	3.8
0870	**1-Hexanol**	0.7	2.2	**4.9**	---	1.7
0904	Heptanal	---	---	---	---	tr
0981	Hexanoic acid	0.4	1.4	1.7	0.4	0.9
0993	2-Pentylfuran	---	tr[2]	tr	tr	1.6
1004	Unidentified	1.4	1.0	1.4	2.5	2.1
1011	***o*-Methylanisole**	0.8	2.3	0.6	**5.3**	0.3
1034	Benzyl alcohol	2.2	1.1	1.2	tr	1.7
1043	Phenylacetaldehyde	0.8	0.6	2.4	0.7	1.0
1056	*o*-Cresol	---	0.3	0.4	1.5	0.2
1072	1-Octanol	2.6	0.8	0.7	---	0.8
1079	Heptanoic acid	0.5	0.2	tr	---	tr
1089	*o*-Guaiacol	0.6	1.1	2.6	1.3	0.9
1105	Nonanal	0.9	0.8	1.0	2.5	1.3
1111	Unidentified	---	0.8	0.3	1.3	tr
1176	**Octanoic acid**	**7.4**	2.4	1.8	0.2	2.3
1193	Methyl salicylate	---	0.1	2.5	---	1.1
1205	Decanal	0.5	0.3	tr	0.6	0.4
1272	Nonanoic acid	0.6	0.6	0.5	0.5	0.4
1312	*p*-Vinylguaiacol	tr	tr	tr	---	0.2
1357	**Eugenol**	1.3	**7.0**	**8.8**	tr	**8.4**
1363	Unidentified sesquiterpene	0.4	0.4	0.2	2.2	0.5
1370	**Decanoic acid**	**24.4**	**5.6**	3.4	0.6	3.4
1398	Vanillin	---	0.7	0.5	2.0	0.3
1410	Dodecanal	tr	tr	---	---	tr
1429	β-Copaene	---	tr	0.2	0.5	---
1454	Geranylacetone	1.4	tr	0.1	tr	0.4
1478	γ-Muurolene	0.5	0.6	0.2	1.0	tr

Table 4. Composition of bark essential oil (continued).

RI[1]	Compound	Experimental tree				
		1	2	3	4	5
		% of essential oil				
1502	α-Muurolene	3.0	0.8	0.3	1.5	tr
1566	**Dodecanoic acid**	**29.2**	**6.5**	**4.2**	0.7	**6.4**
1580	***ar*-Turmerol**	1.0	0.2	0.2	**6.0**	1.1
1674	Cadalene	---	0.5	0.2	0.3	1.4
1766	Tetradecanoic acid	1.8	1.4	1.5	1.6	2.3
1862	Pentadecanoic acid	0.5	0.5	0.5	0.9	0.7
1960	**Palmitic acid**	**8.8**	**12.6**	**13.2**	**43.7**	**21.6**
2100	Heneicosane	2.0	1.1	0.7	tr	1.1
2130	**Linoleic acid**	0.7	3.5	2.4	**9.5**	**8.8**
2136	Oleic acid	---	1.1	0.7	3.0	2.8
2300	Tricosane	---	tr	tr	---	tr
2400	Tetracosane	---	0.5	---	tr	---
2500	Pentacosane	tr	0.5	0.2	---	tr
2600	Hexacosane	---	0.2	tr	---	---
2700	**Heptacosane**	2.5	**24.3**	**19.6**	**4.8**	**13.0**
2830	Squalene	0.6	1.0	1.3	2.3	1.3
2900	Nonacosane	---	3.2	2.3	0.7	1.2
	Benzenoid aromatics	5.9	13.1	18.9	10.8	14.0
	Paraffin hydrocarbons	4.5	29.8	22.7	5.6	15.3
	Monoterpene hydrocarbons	---	---	---	---	---
	Oxygenated monoterpenoids	---	---	---	---	---
	Sesquiterpene hydrocarbons	5.3	2.3	1.4	5.6	2.3
	Oxygenated sesquiterpenoids	1.0	0.2	0.2	6.0	1.1
	Fatty-acid-derived compounds	80.4	51.2	53.8	65.2	60.8
	Others/Unidentified	2.4	2.5	1.7	4.5	5.2
	Total Identified (45)	98.2	97.8	98.1	94.1	97.3

[1]RI = Retention Index determined with respect to a series of normal alkanes on an HP-5ms column.

[2]tr = trace (< 0.05%).

[3]Abundant components indicated in **boldface**.

In our study, all three *B. nigra* essential oils exhibited phytotoxicity against both lettuce (*L. sativa*) and perennial ryegrass (*L. perenne*) (Table 5). The leaf essential oil was the most allelopathic and inhibited seed germination of *L. sativa* and *L. perenne* with an IC_{50} = 1480 and 1120 μg/mL, respectively. The *B. nigra* leaf oil also significantly inhibited root and shoot growth of *L. sativa* at 500 μg/mL. Tworkoski (2002) had reported that commercially available *B. nigra* (sweet birch) essential oil demonstrated only marginal allelopathic activity, based on electrolyte leakage, against dandelion (*Taraxacum officinale*). Similarly, Wilson et al. (1997) reported that commercial *B. nigra* oil showed marginal antifungal activity.

Sweet birch, however, generally refers to *B. lenta* (Lamson, 1990), as opposed to *B. nigra*, and we could not locate a commercial source of the *B. nigra* essential oil despite an extended search. Neither of the above publications reported the essential oil compositions of their *B. nigra*, so the identification of the plant source for the oils remains in doubt, but was likely *B. lenta*. Neither Aroma Vera (Culver City, CA) nor Frontier Natural Products (Norway, IA), commercial sources of essential oils, currently offer birch oil. An analysis of a commercially available sample of sweet birch (*B. lenta*) essential oil (New Directions Aromatics, Ontario, Canada) in our laboratory, noted this oil is composed exclusively of methyl salicylate.

Table 5. Allelopathic effects *B. nigra* oil on lettuce seedlings.

Tissue extract	Test plants	Seed germination	Seedling growth	
			Radicle	Hypocotyl
(μg/mL)	*Lactuca* sp.	(% inhibited)[a]	(% of control)	
Buds	*L. sativa*	71.6	58.4[b]	22.1[b]
2000	*L. perenne*	53.3	53.5[b]	0[b]
Leaves				
500	*L. sativa*	15.0	88.8[d]	53.5[b]
	L. perenne	15.0	>100	>100
1000	*L. sativa*	20.0	82.7[c]	48.3
	L. perenne	36.7	91.0[e]	70.4[e]
2000	*L. sativa*	73.3	31.1	12.8
	L. perenne	100	---	---
Bark				
1000	*L. sativa*	16.7	82.4[d]	>100
	L. perenne	23.3	99.2	>100
2000	*L. sativa*	83.3	77.8[d]	>100
	L. perenne	36.7	81.8[e]	>100
4000	*L. sativa*	96.7	54.0[b]	>100
	L. perenne	41.7	67.4[b]	>100

a = Decrease in germination as compared with 100% control germination.
b = Highly significantly different from control, $P \leq 0.001$.
c = Highly significantly different from control, $P \leq 0.005$.
d= Significantly different from control, $P \leq 0.05$.

The leaf essential oil of *B. nigra*, but not the bud or bark oils, exhibited insecticidal activity against red imported fire ants (*Solenopsis invicta* × *richteri*) (data not shown). The insecticidal activity can be attributed to the major components in the leaf oil. (2*E*)-Hexenal has been shown to be insecticidal against *Anoplolepis longipes*, *Culex quinquefasciatus*, *Sitotroga cerealella*, (Gunawardena and Herath, 1992), *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus granarius*, *Sitophilus oryzae*, and *Cryptolestes ferrugineus* (Hubert *et al*., 2008); linalool is insecticidal to *Culex pipiens* (Traboulsi et al., 2002), *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* (Chang *et al*., 2009); and eugenol has demonstrated toxicity toward *Periplaneta americana* (Ngoh *et al*., 1998), *Sitophilus zeamais*, and *Tribolium castaneum* (Huang *et al*., 2002). *B. nigra* leaf oil was also the most nematocidal of the oils on *C. elegans* (LC_{50} = 457 μg/mL), while all three *B. nigra* essential oils were appreciably toxic toward brine shrimp (*Artemia salina*) with LC_{50} values of approximately 20 μg/mL (data not shown).

All three *B. nigra* essential oils were somewhat antibacterial toward *B.* cereus, with the leaf oil most active (MIC = 156 μg/mL) (Table 6). The bud essential oil demonstrated antifungal activity against both *C. albicans* and *A. niger* (MIC = 313 μg/mL), most likely due to the major component eugenol (Boonchird and Flegel, 1982; Moleyar and Narasimham, 1986; Pinto *et al*., 2009).

Table 6. antimicrobial activity of *B. nigra* essential oils.

Microbe	Essential oil (MIC, μg/mL)		
	Buds	Leaves	Bark
B. cereus	625	156	625
S. aureus	1250	1250	1250
E. coli	1250	625	625
P. aeruginosa	1250	1250	1250
C. albicans	313	1250	1250
A. niger	313	625	625

In summary, the essential oils from the twigs and buds, the leaves, and the inner bark of *B. nigra* are biologically active in several bioassays. The chemical compositions of the essential oils and the observed biological activities are consistent with traditional uses of this tree.

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