JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Variation of Chemical Composition of the Lipophilic Extracts from Yellow Birch (*Betula alleghaniensis*) Foliage

JEAN-MICHEL LAVOIE AND TATJANA STEVANOVIC*

Faculté de Foresterie et de Géomatique, Département des sciences du bois et de la forêt, Université Laval, Québec, Canada G1K 7P4

The occurrence of biologically active compounds identified for the first time in the lipophilic extracts of yellow birch (*Betula alleghaniensis* Britt.) foliage led to the quantification of the seasonal variation of their concentrations. Yellow birch foliage was collected from late June until late September 2003 in two different regions of Quebec. The extraction yields using hexane as a solvent were determined, and the extracts were analyzed by GC-MS to identify their molecular composition. In terms of both extraction yields and the concentration of the targeted molecules present in the extracts, mid-September has been determined as the best time to collect foliage samples. A total of 14 constituents were identified in these extracts. This is the first report of the presence of all of these constituents in yellow birch foliage and of some of them in the genus *Betula*. The most important compounds identified in yellow birch foliage extracts are triterpene squalene and aliphatic hydrocarbon tetracosan, aliphatic alcohol phytol, fatty acids hexadecanoic and octadecanoic, pentacyclic triterpenes α- and β-amyrin, and phytosterol stigmast-5-en-3-ol.

KEYWORDS: ***Betula alleghaniensis*; foliage; lipophilic extracts; seasonal variation; geographical variation; bioactive triterpenes and phytosterols**

INTRODUCTION

The announced upcoming global oil crisis will deeply affect the living habits of industrialized countries. The problems generated by the future lack of oil will affect not only the motor but also the plastics, cosmetics, and pharmaceuticals industries. One of the possibilities to provide bioactive molecules for the latter two industries would be a complete utilization of forest resources. Yellow birch is a common tree in North America, growing from the northern United States to southern Canada. It is the emblem tree of Quebec and the second most important hardwood species for its wood industry, which processes nearly 1 200 000 m^3 of yellow birch wood annually (data for 2003). Its forest exploitation generates huge quantities of different types of residues: bark, small branches, and foliage. Many biologically active compounds have been identified in the bark of different birch species (*1*). Previous work (*2*−*4*) has confirmed the presence of pentacyclic triterpenes with lupane skeletons in yellow birch (*Betula alleghaniensis* Britt.) bark. Here we report the results of a study of the chemical constituents of the lipophilic extracts of yellow birch foliage as well as their variation during the growing season and the influence of the site of sampling. There has been little work done on this subject. A partial evaluation of the phenolic content of birch foliage has been conducted by Baldwin et al. (*5*). They studied the condensed tannins, total phenolics, and protein binding (astringency) capacity of the extracts from yellow birch (*B. alleghaniensis*) and sugar maple (*Acer saccharum*). They did not find any significant variation in the composition of these extracts due to geographical location of the sampling site, but they did find significant seasonal changes in the chemical composition of the extracts. Comparable studies have been done on phenolic concentrations in the foliage of the European birches, *Betula pubescens* (*6*−*8*) and *Betula pendula* (*7*). Many tetracyclic dammarane-type triterpenes have been identified in birch foliage as in a study of *Betula mandschurica* foliage (*9*). Research on other birch species, *B. pendula* and *Betula ermanii* (*10*, *12*), *Betula kelleriana* (*11*), and *Betula nana* (*12*), also confirmed the presence of dammarane-type triterpenoids in the foliage.

The influence of the time of sampling has been studied previously for the triterpenoid betulinol in the bark of *B. pendula* and *B. pubescens* (*13*), and no significant variation with the season was found. The seasonal variation of the extractive composition of foliage has been studied for the hydrolyzable tannins in the foliage of *B. pubescens* (*14*). The results of that work showed that the concentration of galloylglucose dropped with the advancement of the season but that the ellagitannins seemed to stay constant until the end of July, when they suddenly increased before dropping again. Nurmi et al. (*8*) studied the low molar mass phenolic compounds in the foliage of the same species. The results of their study showed that the variation of low molecular weight polyphenols was hardly noticeable during the green period of the leaves. Salminen et al. examined the seasonal variation of hydrolyzable tannins in

* Author to whom correspondence should be addressed [fax (418) 656-3177; telephone (418) 656-2131, ext. 7337; e-mail tatjana.stevanovic@sbf.ulaval.ca].

10.1021/jf050301y CCC: $30.25
Published on Web 05/19/2005

the foliage of other Finnish species of birch, *B. nana*, *B. pendula*, and another variety of *B. pubescens* (*15*). Their results confirmed the significant seasonal variation of some hydrolyzable tannin concentrations within these species, and they were able to relate these to the biosynthetic pathways of the targeted compounds. The seasonal variation of carotene, chlorophyll, and ascorbic acid in the foliage of *Betula alba*, *Betula fruticosa*, and *B. pendula* has been studied in Russia (*16*). Molecules such as ascorbate are present in higher concentrations at the very beginning and at the end of summer and are at their lowest in June. Finally, there have been two investigations on the seasonal variation of chemical components as available carbohydrate, crude fiber, hemicellulose, holocellulose, lignin, polyphenols, N, P, ash K, Ca, and Mg in the leaves of yellow birch (*17*, *18*). The main goal of our research was to investigate the effects of seasonal and geographical variation on the chemical composition of yellow birch foliage lipophilic extracts. Furthermore, after the identification of the molecular composition of these lipophilic extracts, we were able to evaluate the best time to collect the foliage to get the optimal concentration of the bioactive molecules.

MATERIALS AND METHODS

Foliage Sampling. Yellow birch leaves were sampled near the town of St-Félix d'Otis in the Saguenay Lac-St-Jean region of Quebec during September 2001. Foliage had been collected from three different trees, and the ages were determined after felling to be 28, 35, and 47 years, with corresponding diameters at breast height of 13, 18, and 15 cm, respectively. Foliage was also sampled weekly near the town of St-Cyrille de Lessard in the Chaudière-Appalache region of Quebec. The foliage was collected from late June until late September 2003 from a single standing tree. The diameter of the tree was 26 cm at breast height. All samples were immediately placed on ice and then frozen at −15 °C until extraction in the laboratory.

Extraction and Solvents. Before extraction, the leaves were dried in an oven at 60 °C for 1 week to eliminate volatile compounds. The samples were then ground to a powder with a laboratory grinder (Cyclotec 1093 sample mix Tecator) without sieving. Sanples of 5−10 g of the ground foliage were extracted for 6 h by Soxhlet, using hexane as a solvent for lipophilic substances. The hexane was of reagent grade HPLC (Aldrich Inc., Whitby, ON, Canada). The solvent was then removed under reduced pressure at 40 °C. The crude extracts were then dried in an oven at 60 °C for at least 24 h to constant mass, for extraction yield calculations purposes. The dry extracts were finally dissolved in 5 mL of pure hexane to perform GC-MS analysis. Five microliters of each extract was injected into a GC-MS (Hewlett-Packard Inc.).

Pure Compounds and GC-MS. The GC-MS used in our experiments is a Hewlett-Packard. The GC was a HP5890 model coupled with a DB-5 column eluted with helium. The column was 30 m long by 0.25 mm wide and was loaded with a 0.25 μm methyl (5% phenyl) silicone packing film (Chromatographic Spec. Inc.). The mass spectrometer was a HP5972 model. The temperature program in the GC core started at 100 °C and then increased at a rate of 25 °C/min until it reached 280 °C. It then increased at a rate of 5 °C/min until a temperature of 325 °C was reached. The temperature of the injector was 225 °C, and the split value was 1:46. The molecular ionization was made by electron impact (EI) at 70 eV. The scanning range was between *m*/*z* 40 and 700, and the temperature source was 162 °C. The chromatographic identifications were performed by comparisons with the mass spectra of the authenticated compounds or by direct comparison to literature mass spectra. Comparison to pure compounds has been made with the previously injected standards at the Laseve laboratory in Chicoutimi, PQ, Canada. The evaluation of the molecular concentration was based on surfaces in the MS-TIC chromatograms.

RESULTS

Extraction Yields. Comparison of the results for the extraction yields obtained from yellow birch leaves collected from trees of comparable ages from two different geographic locations, St-Félix d'Otis (48° 16′ 24″, −70° 37′) and St-Cyrille de Lessard (47° 3′ 59″, −70° 7′ 29″), at approximately the same time of year confirmed that no substantial differences in the amounts of extracts were found (**Tables 1** and **2**).

Table 1. Net Hexane Extraction Yields, Temperature, and Amount of Precipitations for Three Foliage Samples Harvested in St-Félix d'Otis on September 17, 2001[a]

age (years)/diameter (cm) of the specimen	temp (°C)/precip (mm)	hexane extraction yield (%)
28/13.05	16.2/0.8	1.26
35/17.83	16.2/0.8	1.68
47/14.96	16.2/0.8	2.10

[a] Total amount of precipitation for the summer of 2001 at this location was 271.8 mm. Maximum temperature was 32.2 °C, and minimum was 0.2 °C.

Table 2. Net Hexane Extraction Yields, Temperature, and Amount of Precipitation for Foliage Samples Harvested at St-Cyrille de Lessard Site during the Summer of 2003[a]

sampling date	temp (°C)/precip (mm)	extraction yield (%)
June 29, 2003	21.9/2.6	1.77
July 5, 2003	21.6/0.6	2.18
July 13, 2003	17.9/1.6	1.66
July 20, 2003	19.4/3.2	1.99
July 31, 2003	20.5/0	0.98
Aug 3, 2003	23.6/0	0.96
Aug 10, 2003	16.8/41.4	1.20
Aug 17, 2003	19.8/0	1.76
Aug 24, 2003	12.8/0	1.01
Sept 1, 2003	15.9/0	1.56
Sept 7, 2003	14.4/0	1.24
Sept 14, 2003	21.1/0	1.75
Sept 19, 2003	17.1/3.2	2.51
Sept 27, 2003	16.1/0	1.97

[a] Total amount of precipitation for the summer of 2003 at this location was 361.2 mm; maximal temperature recorded was 29.2°C and minimal, 4.2°C.

The extraction yields obtained from the foliage collected from the oldest tree at the St-Félix d'Otis site in September 2001 seemed to match the extraction yields determined from foliage collected during the same period in September 2003 at the St-Cyrille de Lessard site (**Tables 1** and **2**). Different patterns of concentration variations were determined for the triterpene and other lipophilic constituents of extracts of yellow birch foliage (**Figures 2** and **3**).

The concentration of α-amyrin remained relatively stable throughout the sampling period and that of β-amyrin decreased steadily to reach zero by the end of the sampling period for the St-Cyrille site, whereas the concentration of squalene reached values of 15 and 25% in August and September, respectively, after which it started to decrease (**Figure 3**). The concentration of betulonic aldehyde was very low throughout the sampling period and then increased abruptly in mid-September to 10% of the extract (**Figure 3**). The concentration of 1,10-undecadiene was ∼1% in the extracts of foliage collected in the beginning of the sampling and slightly increased with progression of time to drop to zero in the beginning of August (**Figure 2**). Similar concentrations were observed for hexadecanoic acid, which represented nearly 5% of the extracts collected at the beginning of sampling and then dropped to zero at the beginning of August and increased again to nearly 2% of the extract by mid-September (**Figure 2**). The concentration of phytol increased from 1% in June to a maximum of 3% at the beginning of

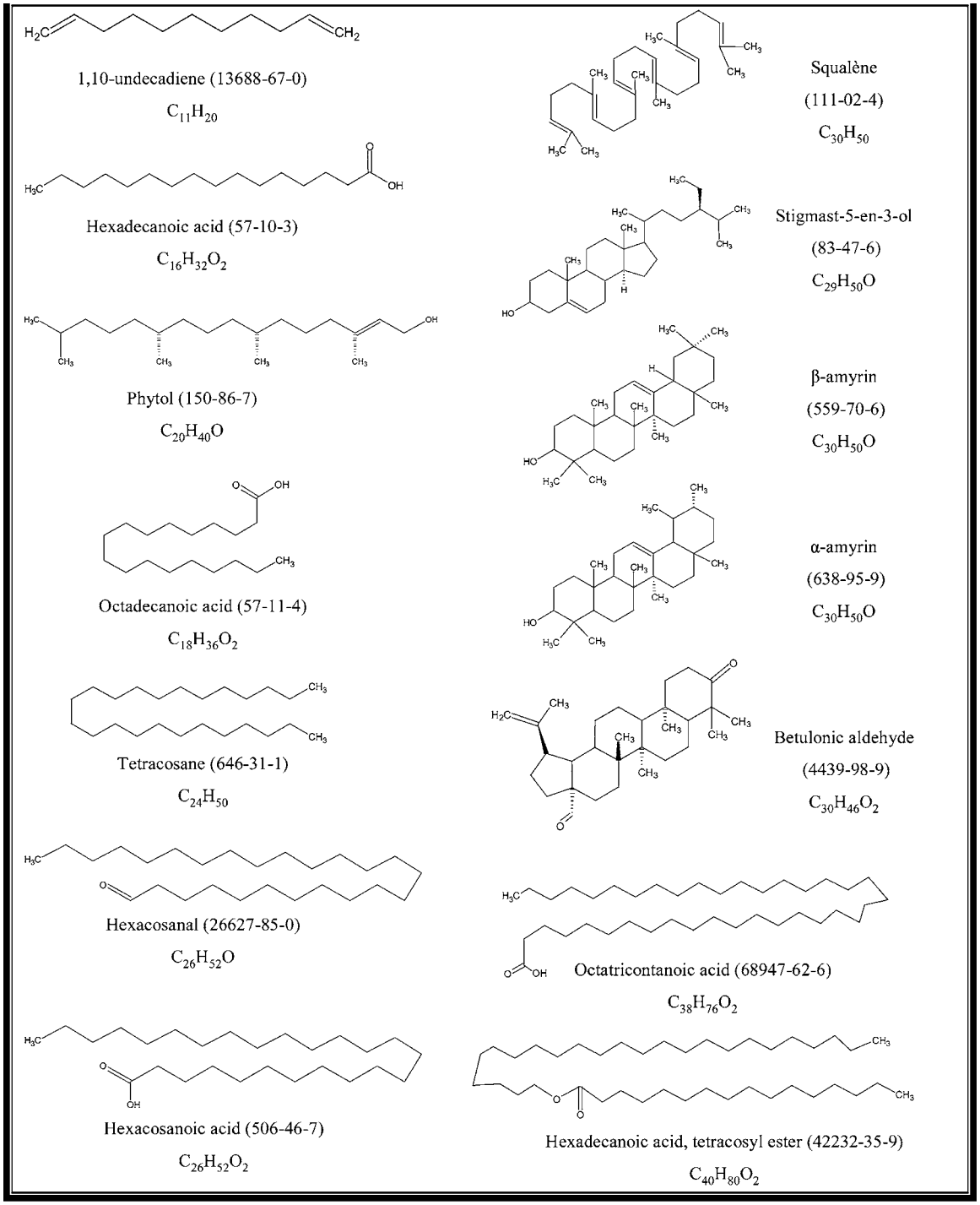

Figure 1. Structures, formulas, and CAS Registry numbers (provided by the author) of the molecules identified in the yellow birch foliage extracts.

September and then dropped to zero (**Figure 2**). The concentration of octadecanoic acid varied between 1 and 4% of the extracts from leaves collected from early June to mid-August and then disappeared completely from the extracts in the second week of August (**Figure 2**). The concentration of tetracosan remained relatively stable at ∼1.5% throughout the sampling season to decrease by mid-August and the second week of September.

Hexacosanal was constantly present in the foliage extracts throughout the sampling period, at concentrations varying from 4 to 6.5% (**Figure 2**). The concentration of hexacosanoic acid remained stable, at 1−2% of the extracts, throughout the sampling period (**Figure 3**). The squalene concentration increased with progression of the season. We determined the concentration of squalene at the beginning of the sampling period to be ∼1% of the extract, to increase to almost 25% of the extract by mid-September, and to decrease again to 10% at the end of the season (**Figure 3**). Contrary to what we found for squalene, the concentration of stigmast-5-en-3-ol decreased through the sampling period, from nearly 20% in late June to 12−13% at the end of the season (**Figure 3**). The concentration of octatricontanoic acid remained steady throughout the sampling period and then dropped to zero at the end of the sampling period (**Figure 3**). Finally, the concentrations of hexadecanoic acid, octacosyl ester, remained high, between 15 and 25% of the extracts, throughout the sampling period, decreasing slightly by the end of sampling period but remaining still at 15% of the extract at the end of the sampling (**Figure 3**).

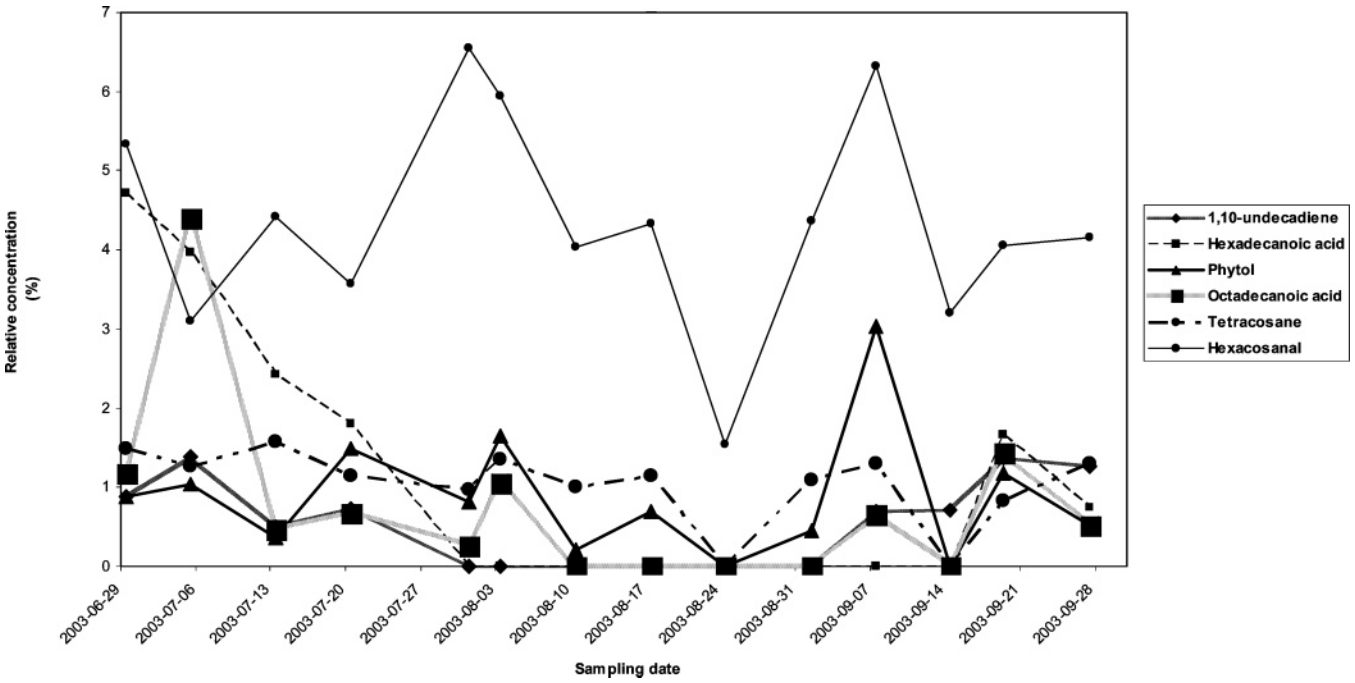

Figure 2. Variation of the relative concentration of the low molecular mass compounds (compounds **I**–**VI**) in yellow birch foliage extracts.

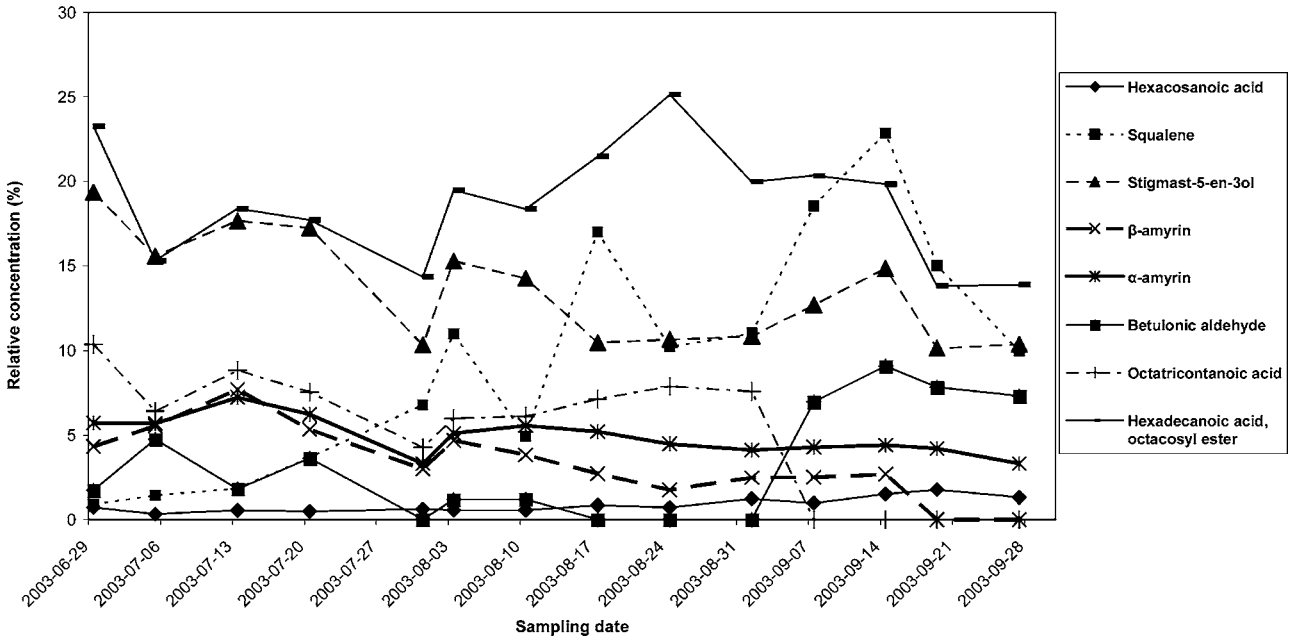

Figure 3. Variation of the relative concentration of the higher molecular mass compounds (compounds **VII**–**XIV**) in yellow birch foliage extracts.

DISCUSSION

Molecular Composition. The results presented in **Table 4** demonstrate that the majority of the compounds (depicted in **Figure 1** and listed in **Table 3**) identified in the extracts of foliage from the St-Félix d'Otis site were confirmed 2 years later in the extracts of foliage from the St-Cyrille de Lessard site. This indicates that it may be a standard molecular composition of yellow birch foliage extracts, regardless of the geographical location of the sampling site.

Compound I (1,10-undecadiene) is not a compound commonly associated with plant tissues in the literature. This constituent has been only once reported from plants, in the essential oils from cantaloupe (*19*).

The concentration of this compound in the extracts of yellow birch foliage is very low, reaching zero 1 month after the beginning of sampling in June (**Figure 2**). A literature survey found no mention of the medicinal properties of 1,10-undecadiene. The presence of this compound in our chromatograms was confirmed through comparisons from literature data on the mass spectrum.

Compound II (hexadecanoic acid) is a constituent commonly associated with plant tissues, often reported in scientific publications. We have determined it to be present in the yellow birch foliage extracts only at the beginning of the summer period, its concentration in the extracts constantly decreasing throughout the sampling period, to disappear completely at the beginning of August (**Figure 2**). The hexadecanoic acid has already been identified in the foliage from native birches of Turkey: *Betula browicziana*, *Betula litwinowii*, *Betula medwedewii*, *B. pendula* and *Betula recurvata* (*20*). It has also been

Table 3. Molecules Identified in Yellow Birch Foliage Extracts from St-Cyrille de Lessard and St-Félix d'Otis

compd	mass spectrum [*m*/*z* (intensity)]	t_R (min)	identification
I	152 (1), 137 (6), 124 (14), 123 (31), 111 (12), 109 (17), 97 (25), 96 (21), 95 (59), 83 (38), 82 (62), 81 (41), 71 (36), 70 (15), 69 (61), 68 (91), 67 (50), 57 (71), 56 (22), 55 (78), 53 (17), 44 (20), 43 (100), 41 (90)	7.834	1,10-undecadiene
II	256 (1), 213 (10), 157 (9), 129 (27), 155 (11), 97 (15), 87 (16), 85 (19), 84 (10), 83 (21), 73 (91), 71 (33), 69 (33), 61 (23), 60 (89), 57 (63), 56 (17), 55 (67), 45 (12), 43 (100), 42 (18), 41 (83)	8.381	hexadecanoic acid
III	296 (1), 126 (4), 123 (18), 111 (6), 95 (12), 83 (14), 82 (10), 81 (22), 72 (6), 71 (100), 70 (14), 69 (25), 68 (17), 67 (10), 57 (33), 56 (16), 55 (31), 43 (46), 42 (6), 41 (39)	9.100	phytol
IV	284 (9), 185 (10), 129 (23), 115 (10), 97 (18), 96 (8), 95 (14), 87 (13), 85 (19), 84 (10), 83 (25), 82 (10), 81 (16), 79 (17), 73 (86), 71 (33), 70 (13), 69 (40), 68 (12), 67 (25), 61 (20), 60 (68), 57 (62), 56 (19), 55 (77), 54 (10), 45 (11), 44 (20), 43 (100), 42 (20), 41 (88)	9.297	octadecanoic acid
V	338 (1), 141 (3), 130 (3), 127 (4), 125 (4), 99 (11), 98 (5), 97 (15), 85 (38), 84 (7), 83 (20), 71 (57), 70 (13), 69 (21), 57 (100), 56 (17), 55 (35), 43 (85), 42 (10), 41 (41)	11.045	tetracosane
VI	380 (1), 155 (2), 139 (3), 127 (4), 125 (7), 113 (7), 112 (5), 111 (15), 99 (11), 98 (8), 97 (31), 85 (37), 84 (11), 83 (34), 82 (15), 71 (57), 70 (18), 69 (36), 68 (13), 57 (100), 56 (24), 55 (51), 43 (89), 42 (11), 41 (43)	12.340	hexacosanal
VII	396 (1), 195 (3), 167 (4), 153 (5), 139 (7), 125 (17), 124 (4), 113 (5), 112 (11), 111 (30), 110 (5), 99 (6), 98 (14), 97 (67), 96 (16), 85 (22), 84 (20), 83 (69), 82 (32), 81 (11), 71 (47), 70 (36), 69 (65), 68 (19), 67 (17), 57 (93), 56 (40), 55 (100), 54 (12), 43 (98), 42 (16), 41 (78)	12.640	hexacosanoic acid
VIII	410 (1), 395 (1), 367 (1), 341 (2), 325 (1), 299 (1), 273 (1), 257 (1), 231 (2), 217 (2), 203 (2), 175 (3), 161 (4), 137 (10), 121 (8), 95 (12), 81 (50), 69 (100), 41 (30), 32 (2)	13.23	squalene
IX	398 (1), 396 (6), 329 (11), 303 (11), 273 (8), 255 (10), 231 (9), 213 (16), 199 (7), 178 (7), 163 (17), 161 (18), 160 (10), 159 (18), 145 (26), 135 (15), 134 (13), 133 (20), 131 (16), 123 (15), 121 (20), 120 (16), 119 (27), 109 (18), 107 (35), 105 (35), 95 (35), 93 (29), 91 (31), 81 (38), 71 (19), 69 (38), 67 (28), 57 (49), 55 (58), 43 (100), 41 (57)	18.062	stigmast-5-en-3-ol
X	426 (1), 231 (2), 220 (2), 219 (19), 218 (100), 205 (18), 204 (12), 203 (57), 189 (16), 149 (9), 148 (5), 147 (8), 137 (10), 136 (10), 135 (16), 133 (13), 123 (10), 121 (15), 119 (19), 109 (22), 107 (21), 105 (21), 95 (30), 94 (15), 93 (21), 91 (18), 81 (27), 79 (19), 69 (35), 67 (19), 55 (44), 43 (24), 41 (37)	18.350	β-amyrin
XI	426 (2), 257 (3), 219 (19), 218 (100), 204 (10), 203 (50), 189 (18), 175 (8), 161 (8), 147 (13), 137 (9), 136 (10), 135 (16), 133 (13), 121 (17), 119 (19), 109 (21), 107 (21), 105 (23), 95 (31), 94 (14), 93 (21), 91 (19), 83 (10), 81 (29), 79 (18), 71 (9), 69 (42), 67 (20), 57 (26), 55 (44), 43 (42), 41 (38)	18.700	α-amyrin
XII	438 (1), 216 (8), 203 (16), 201 (13), 175 (23), 173 (18), 163 (10), 161 (19), 159 (15), 149 (16), 148 (13), 147 (28), 145 (18), 135 (27), 134 (16), 133 (31), 131 (14), 123 (24), 122 (13), 121 (35), 119 (38), 109 (35), 108 (12), 107 (48), 105 (37), 97 (13), 96 (11), 95 (60), 94 (12), 93 (50), 91 (41), 83 (30), 81 (49), 77 (19), 71 (17), 69 (93), 68 (10), 67 (45), 57 (28), 55 (100), 43 (62), 41 (76)	19.700	betulonic aldehyde
XIII	564 (3), 257 (31), 229 (38), 139 (4), 125 (10), 111 (20), 98 (11), 97 (40), 96 (10), 85 (30), 84 (14), 83 (45), 82 (18), 81 (10), 73 (13), 71 (50), 70 (17), 69 (46), 57 (100), 56 (22), 55 (59), 43 (92), 42 (12), 41 (38)	28.490	octatricontanoic acid
XIV	592 (2), 258 (10), 257 (58), 139 (4), 129 (7), 125 (10), 111 (20), 98 (10), 97 (40), 85 (31), 84 (13), 83 (44), 82 (17), 81 (10), 73 (14), 71 (51), 70 (17), 69 (45), 68 (13), 67 (11), 61 (11), 57 (100), 56 (21), 55 (56), 44 (3), 43 (90), 42 (12), 41 (36)	34.980	hexadecanoic acid, octacosyl ester

identified in the leaves of a *B. pendula* growing in Russia (*21*−*23*) and in the extracts obtained from the inner bark of *Betula verrucosa* (*24*). Finally, this compound has been previously reported from yellow birch wood (*25*, *26*). To the best of our knowledge, this is the first report on its presence in the foliage of yellow birch. Specific pharmacological properties have not been attributed to this molecule, despite its frequent occurrence in biologically active extracts. For instance, palmitic (hexadecanoic) acid has been identified in the extracts of the medicinal plant *Sideritis taurica*, which have been proved to possess analgesic, anti-inflammatory, antiulcerogenic, and antihyperglycemic activities (*27*). It has also been reported in the extracts from *Plantago albicans* (*28*), *Inula montana* (*29*), *Andrographis paniculata* (*30*), *Pentanisia prunelloides* (*31*), and *Dalbergia sissoo* (*32*), some of which are medicinal plants. We have identified it in yellow birch hexane extracts by comparison to authentic compound.

Compound III (phytol) is also widespread in plant tissues. Its concentration in yellow birch foliage extracts seems to remain constant at 2% throughout the season, except for a little variation in September when its concentration reached 3% of the extract. This increase could be linked to the chlorophyll and the late seasonal variation of the pigmentation of the leaves. The breaking of the phytol bond in chlorophyll of the foliage in fall has already been proposed as an explanation for the larger amounts of this constituent in foliage extracts in autumn (*33*). This molecule has been identified in the foliage of Turkish birches studied by Demirci (*20*). Even though this compound has been often found in medicinally potent plant extracts, no extended clinical studies have been performed on it. The antiinflammatory and hepatoprotective properties of *I. montana* extracts (*29*) have been long exploited in traditional medicine. This molecule is also closely related to the extracts of *Coleus parvifolius* with HIV-1 integrase inhibitory properties (*34*). Phytol presence was confirmed in the extracts of *Ballota pseudodictamnus*, having antimicrobial activity (*35*), and those of *Morinda citrifolia* with antitubercular properties (*36*), which inhibit the mutagenesis and tumor cell growth (*37*). It was identified in our extracts by comparison with pure phytol.

Table 4. Comparison of the Chemical Composition of the Foliage Extracts of Samples Collected at Comparable Times from St-Cyrille de Lessard (September 14) and St-Félix d'Otis (September 17)

compd	St-Cyrille de Lessard (%)	St-Félix d'Otis (%)
1,10-undecadiene	1.27	0.86
hexadecanoic acid	0.75	0.44
phytol	0.53	1.62
octadecanoic acid	0.53	<0.1
tetracosane	1.30	0.83
hexacosanal	4.16	8.13
hexacosanoic acid	1.31	2.34
squalène	9.97	10.81
stigmast-5-en-3-ol	10.37	9.55
β-amyrin	2.69	1.92
α-amyrin	3.32	34.16
betulonic aldehyde	7.33	1.57
octatricontanoic acid	<0.1	2.62
hexadecanoic acid, octacosyl ester	13.89	7.04

Compound IV (octadecanoic acid) has been determined in small concentrations in the extracts of the yellow birch foliage studied here, throughout the sampling season (**Figure 2**). This acid, commonly accompanied in plants by hexadecanoic acid (compound **II**) has already been reported from buds and leaves of *Betula* species (*22*, *23*, *39*), in pulp waste of *Betula platyphylla* (*39*), and in the inner bark of *B. verrucosa* (*24*) as well as in wood of *B. alleghaniensis* (*25*, *26*). To the best of our knowledge this is the first report of its presence in the leaves of yellow birch. Given the fact that this acid is commonly accompanied by palmitic acid in plant tissues, it is normal that these compounds are related to the same medicinal plants.

The extracts of *S. taurica*, which also contain this acid, have analgesic, anti-inflammatory, antiulcerogenic, and antihyperglycemic activities (*27*), as is the case with the extracts of *P. albicans* (*28*), whereas the extracts of *Plocamium telfairiae* have antitumor activity (*40*) and finally those from *Dalbergia sissoo* have anti-inflammatory, antipyretic, analgesic, and estrogen-like activities (*32*). It was identified in our extracts by comparison to authentic compound.

Compound V (tetracosane) is one of the rare compounds the concentration of which remained relatively stable (~1% of the extracts) throughout the sampling period (**Figure 2**). It has been reported previously only in the bark of *B. platyphylla* (*41*). It is a common constituent of *Rhizophora* species (*R. mangle*, *R. racemosa*, and *R. harrisonii*) (*42*) and of the plant *Myrthus communis* (*43*). This compound is commonly found together with palmitic and stearic acids in the extracts of plant tissues with medicinal value. Coincidently, its presence was also confirmed in the *S. taurica* extracts with important biological properties already discussed (*27*). Its presence is also commonly associated with the antipyretic, antiinflammatory, and hepatoprotective activity of the aerial part extracts from *I. montana* (*29*), for the analgesic, tranquilizing, and progesterone-like effects of *Lagonychium farctum* (*44*), for the hypoglycemic and hypocholesterolemic activity of *Tamarindus indica* (*45*), and for the improvement in the blood cholesterol, triglycerides, and creatinine in rats by the extracts from the seeds of *Balanites aegyptiaca* (*46*). Its presence in yellow birch foliage extracts was confirmed by comparison with literature data on mass spectra.

Compound VI (hexacosanal) was present in yellow birch foliage extracts at concentrations varying between 3 and 7% for most of the sampling period and dropped to a minimum of 1.5% during the third week of August (**Figure 2**). This aliphatic aldehyde, closely associated with plant waxes, is to the best of our knowledge for the first time reported from a birch tissue. This constituent has been identified previously in apples (*47*), in many species of the genus *Euphorbia* growing in Greece (*48*), in olive oil (*49*), in *Thymus* (*50*), and among trees in the foliage of *Quercus robur* (*51*) as well as in the foliage of many species of aspen (*52*). This molecule has not been associated with any specific medicinal properties in the literature. Its presence in our extracts was confirmed by comparison with literature data on the mass spectrum.

Compound VII (hexacosanoic acid) was determined to be present at a low concentration of ~1% throughout the sampling period (**Figure 3**). It has been previously reported from the extracts of *Rhododendron canescens* (*53*) and from five different species of the genus *Arachis*: *A. grandulifera*, *A. batizocoi*, *A. ipaensis*, *A. chacoense*, and *A. paraguariensis* (*54*). There are few studies associating this compound with medicinal properties. Ansari et al. (*55*) identified this molecule in the extracts of *Eupatorium adenophorum*, the administration of which was associated with an increase of motor activity. Positive effects of this compound in treating adrenoleukodistrophy were demonstrated in experiments with rats (*56*). Its presence in yellow birch extracts was confirmed by comparison with literature data on the mass spectrum.

Compound VIII (squalene) is a noncyclic triterpene and is a precursor in the biosynthetic route to the cyclic triterpenoids (*57*). Its concentration is low in the beginning of the sampling period but gains importance during the season; squalene becomes one of the major constituents of the extracts by the end of the season, reaching the concentration of 25% of the yellow birch foliage extract by mid-September (**Figure 3**). We have already identified this molecule in the wood of yellow birch (*58*); this molecule has never been reported in any other tissue of yellow birch. This compound has already been identified in the wood of another birch species, *B. verrucosa* (*24*, *59*), and also in the wood of the linden tree, *Tilia vulgaris* (*60*).

The presence of squalene in olive oil is often related to its nutraceutical value and to the benefits for the health upon its consumption (*61*). As with palmitic and stearic acid, the presence of squalene in *S. taurica* extracts can be related to the analgesic, anti-inflammatory, antiulcerogenic, and antihyperglycemic effects of this herbal tea commonly used in Turkey (*27*). It is also related to the antibacterial properties of *Solanum elaeagnifolium* (*62*). Its presence in our extracts was confirmed by injection of the pure compound.

Compound IX (stigmast-5-en-3-ol) has also been identified in the wood of yellow birch by our research team (*58*). It is a very important constituent of the yellow birch foliage extracts studied here, its concentration varying from 10 to 20% of the extract. This compound has also been reported in wood extracts of *Dichrostachys cinerea* (*63*), *Eucalyptus urophylla* (*64*), *Markhamia zanzibarica* (*65*), and *Stemmadenia donnell-smithii* (66). The use of phytosterols in preventive medicine is nowadays very popular. Many studies performed with phytosterols in recent years have proven the medicinal potential of these compounds.

A recent patent proposed the use of phytosterols and particularly stigmast-5-en-3-ol as anti-inflammatory agents and in preventing cardiovascular diseases (*67*). Stigmast-5-en-3-ol was determined in our extracts by comparison with the pure compound.

Compound X (β-amyrin) was identified in yellow birch internal bark extracts (*2*) and, furthermore, in specimens of yellow birch foliage collected previously (in 2001) from the

St-Félix d'Otis site. This molecule is present at low concentrations (between 2 and 7%, dropping to zero at the end of sampling period) but still conferring nutraceutical properties to the leaves (*69*). This compound has never been reported before in another *Betula* genus. It has been identified in the foliage from many species of *Rhizophora* (*42*), in the leaves of rhododendron (*54*), and in the foliage of many corn plants (*69*). As is the case for the majority of the constituents of yellow birch foliage extracts, β-amyrin is often found in medicinal plants. The sedative effect of *Maytenus forsskaoliana* (*70*), the antispasmodic, antiarrhythmic, and anticholinergic properties of *Salvadora persica* (*71*), the anti-inflammatory, antipyretic, analgesic, and estrogen-like activities of *D. sissoo* (*32*), the analgesic activity of *Aleurites moluccana* (*72*), and the anti-inflammatory, analgesic, and moderate antipyretic activity of *Cleome amblyocarpa* (*73*) are all associated with the medicinal properties of the amyrin-type triterpenoids (*74*, *75*). β-Amyrin was determined in our extracts by comparison, following the injection of the pure compound.

Compound XI (α-amyrin) is a compound that we are reporting for the first time from any tissue of any species belonging to the genus *Betula*. This molecule is found in relatively stable concentrations, up to 7% of the crude extract (**Figure 3**). The compound has been proven to possess therapeutically interesting features (*68*). It is widely distributed in the genus *Rhizophora* (*42*) and was also found in the foliage of the corn plant (*69*).

Its pharmacological value has been observed in many plant extracts. The analgesic, anti-inflammatory, antiulcerogenic, and anti-hyperglycemic activities of *S. taurica* (*27*) are associated with the content of α-amyrin in this herbal tea. This compound is also closely associated with the anti-inflammatory and analgesic activities of *Himatanthus sucuuba* (*76*) and to the well documented antilipoxygenase and antiarthritic activity of the amyrin triterpenoids (*74*, *75*). α-Amyrin was determined in our extracts by comparison, following the injection of the pure compound.

Compound XII (betulonic aldehyde) has already been identified in bark extracts from the same species (*B. alleghaniensis*) (*2*, *3*). We found it in low concentrations during most of the summer season until September, when its concentration almost doubled and remained at the same level until the end of the sampling period (**Figure 3**). This molecule has also been found in the bark of other birch species: in the outer bark of *B. nigra* (*77*) and in the bark of *B. pendula* and *B. pubescens* (*78*). It has been identified only once in the foliage of forest trees, in the leaves of the Mediterranean oak *Quercus suber* (*79*). We have not been able to find a citation linking this compound to any specific medicinal property. Its presence in yellow birch foliage extracts was determined by comparison to pure compound.

Compound XIII (octatricontanoic acid) is a long-chain aliphatic acid, which is possibly related to the waxy appearance of the yellow birch foliage. Its concentration is found to be relatively constant throughout the summer season until the beginning of September, when it suddenly drops. This observation could be related to the imminent death of the foliage. The identification of this molecule in plants has rarely been reported in the literature.

It was found in sunflower oil (*80*), in the *Amaranthus palmeri* (*81*), and in the foliage of *Breynia rhamnoids* (*82*). We have not found any citation in the literature associating the presence of this particular molecule with any specific medicinal property. Its presence in yellow birch foliage extracts was confirmed by comparison with literature data on the mass spectrum.

Compound XIV (hexadecanoic acid, octacosyl ester) is a long-chain aliphatic plant wax. It appears to be a very important constituent of the lipophilic extracts of yellow birch foliage, its concentration varying from 15 to 25% of the extracts throughout the sampling season. This ester has previously been identified in the fruit of *Rubus idaeus* (*83*), in the olive tree *Olea europaea* fruit (*84*), in the foliage of green tobacco (*85*), and in the foliage of *Clarkia elegans* (*86*). No citation in the literature was found regarding the presence of this particular molecule with any specific medicinal properties. Its presence in yellow birch foliage extracts was confirmed by comparison with literature data on the mass spectrum.

Our study on the chemical composition of the yellow birch foliage lipophilic extracts is the first of its kind on this species. We were able to identify in these extracts six constituents that have never been previously reported in the tissue of any birch species: octatricontanoic acid, octacosyl ester of hexadecanoic acid, α-amyrin, hexacosanoic acid, hexacosanal, and 1,10-undecadiene (**Table 4**). The 14 constituents we have identified in the hexane extracts of the yellow birch foliage collected from two geographically remote locations in Quebec are reported for the first time for yellow birch foliage. The observed variation in concentrations of these compounds in the extracts from two sites could be attributed both to the climate or genotypic differences between the two geographically distant locations and to the differences due to time of sampling (in 2001 and 2003).

We determined the concentrations of 1,10-undecandiene and hexadecanoic acid in the extracts of foliage samples from St-Cyrille de Lessard to be half the value of those found for the extracts of foliage from St-Félix d'Otis. The same is true for the compounds octadecanoic acid, tetracosane, betulonic aldehyde, octatricontanoic acid, and hexacosanoic acid, octacosyl ester. The rest of the foliage extract constituents demonstrated quite the opposite tendency, being present in much higher concentrations in foliage collected from St-Cyrille de Lessard, except for α-amyrin, which was found in the extracts at rates 10 times higher in the St-Félix d'Otis foliage. Despite the observed variation, we were able to confirm the constant presence for all 14 identified constituents of yellow birch foliage extracts studied. The majority of these 14 yellow birch foliage constituents are biologically active compounds. A recent work on *B. platyphylla* (*1*) has shown that the genus *Betula* could be considered as a genus having species with medicinal properties. The fact that squalene, a well-known immunostimulant (*58*), and α-amyrin and β-amyrin, with well-documented therapeutic properties (*69*), are found together in the foliage extracts of *B. alleghaniensis* allows us to anticipate the potential application of foliage crude extracts as nutraceutical products or as a chemical source for bioactive compound isolations. It is well-known that the Potawatomi Indians were using the wintergreen taste of the yellow birch foliage to mask the taste of unappetizing medication and also to combine it with maple extracts for a beverage.

Three different skeleton types of pentacyclic tritepenes are present in our extracts: lupane (betulonic aldehyde), oleanane (β-amyrin), and ursane (α-amyrin). The dammarane-type triterpenes determined in the foliage of some European birches (*9*−*12*) do not seem to be present in the yellow birch foliage.

Most of the identified constituents of our extracts are bioactive and are commonly found in medicinal plants and/or olive oils, which makes yellow birch foliage an interesting source of

nutraceutical or pharmacological molecules. As an example, the biologically active compounds hexadecanoic acid, octadecanoic acid, tetracosane, squalene, and α-amyrin were also identified in the Mediterranean medicinal plant *S. taurica* (*27*, *87*). Therefore, we anticipate a potential application of yellow birch foliage extracts in the food, pharmaceutical, and cosmetic industries. All of these results lead us to propose that yellow birch should be considered a medicinal plant which merits further pharmacological study. The potential use of yellow birch as a source of biologically active components could add value to the transformation of this important wood species widely used by the wood industry of Quebec.

ACKNOWLEDGMENT

We thank M. Gérald Lavoie and Lucille Gaudreault for help with foliage sampling in St-Cyrille de Lessard, M. Jean-Guy Corneau for authorization to take the samples in his forest, and Dr. André Pichette and Hélène Gagnon (Laboratoire Laseve) for work done on the GC-MS in the LASEVE laboratory.

LITERATURE CITED

(1) Ju, E. M.; Lee, S. E.; Hwang, H. J.; Kim, J. H. Antioxidant and anticancer activity of extract from *Betula platyphylla* var. *japonica*. *Life Sci.* **2004**, *74*, 1013−1026.

(2) Cole, B. J. W.; Bentley, M. D.; Hua, Y.; Bu, L. Triterpenoids constituents in the outer bark of yellow birch (*Betula alleghaniensis*). *J. Wood Chem. Technol.* **1991**, *11* (2), 209−223.

(3) Seshadri, T. R.; Vedantham, T. N. C. Betulaceae. Chemical examination of the barks and heartwoods of *Betula* species of American origin. *Phytochemisty* **1971**, *10*, 897−898.

(4) Habiyaremye, I.; Stevanovic-Janezic, T.; Riedl, B.; Garneau, F.-X.; Jean, F.-I. Pentacyclic triterpene constituents of yellow birch (*Betula alleghaniensis*) bark from Quebec. *J. Wood Chem. Technol.* **2002**, *22* (2), 83−91.

(5) Baldwin, I. T.; Schultz, J. C.; Ward, D. Patterns and sources of leaf tannin variation in yellow birch (*Betula alleghaniensis*) and sugar maple (*Acer saccharum*). *J. Chem. Ecol.* **1987**, *13* (5), 1069−1078.

(6) Loponen, J.; Lempa, K.; Ossipov, V.; Kozlov, M. V.; Girs, A.; Hangasmaa, K.; Haukioja, E.; Pihlaja, K. Patterns in content of phenolic compounds in leaves of mountain birches along a strong pollution gradient. *Chemosphere* **2001**, *45* (3), 291−301.

(7) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J. P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(8) Nurmi, K.; Ossipov, V.; Haukioja, E.; Pihlaja, K. Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (*Betula pubescens* ssp. *tortuosa*). *J. Chem. Ecol.* **1996**, *22* (11), 2023−2040.

(9) Malinovskaya, G. V.; Novikov, V. L.; Denisenko, V. A.; Uvarova, N. I. New triterpene from *Betula mandschurica* leaves. *Khim. Prir. Soedin.* **1980**, *3*, 346−351.

(10) Pokhilo, N. D.; Denisenko, V. A.; Uvarova, N. I. New triterpenes from leaves of *Betula pendula* and *Betula ermanii*. *Khim. Prir. Soedin.* **1991**, *1*, 145−146.

(11) Pokhilo, N. D.; Makhnev, A. K.; Uvarova, N. I. Triterpenoids in leaves of Ural and Altai birches in relation to their chemotaxonomy. *Khim. Prir. Soedin.* **1990**, *2*, 278−279.

(12) Pokhilo, N. D.; Malinovskaya, G. V.; Makhan'kov, V. V.; Denisenko, V. A.; Uvarova, N. I. Triterpenoids from the leaves of Siberian birch species *Betula nana* and *B. exilis*. *Khim. Prir. Soedin.* **1985**, *3*, 352−356.

(13) Semenchenko, V. F.; Kudrin, S. V. The content of betulinol in the external bark of birch (*Betula*). *Farmatsiya (Moscow, Russ. Fed.)* **1992**, *41* (4), 24−27.

(14) Salminen, J. P.; Ossipov, V.; Haukioja, E.; Pihlaja, K. Seasonal variation in the content of hydrolyzable tannins in leaves of *Betula pubescens*. *Phytochemistry* **2001**, *57* (1), 15−22.

(15) Salminen, J.-P.; Ossipov, V.; Pihlaja, K. Distribution of hydrolysable tannins in the foliage of Finnish birch species. *Z. Naturforsch., C: J. Biosci.* **2002**, *57* (3/4), 248−256.

(16) Zubareva, N. N.; Aniskina, A. A. Carotene, chlorophyll and ascorbic acid in arboreal foliage of the birch. *Prodovol. Kormovye Resur. Lesov Sib.* **1983**, 18−24.

(17) Ricklefs, R. E.; Matthew, K. K. Chemical characteristics of the foliage of some deciduous trees in southeastern Ontario. *Can. J. Bot.* **1982**, *60* (10), 2037−2045.

(18) Hoyle, M. C. Variation in content of microelements in yellow birch (*Betula alleghaniensis*) foliage due to season and soil drainage. *Soil Sci. Soc. Am. Proc.* **1969**, *33* (3), 458−459.

(19) Beaulieu, J. C.; Grimm, C. C. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J. Agric. Food Chem.* **2001**, *49*, 1345−1352.

(20) Demirci, B.; Baser, K. H. Essential oils from the buds of *Betula spp*. growing in Turkey. *Flavour Fragrance J.* **2003**, *18* (2), 87−90.

(21) Rodionov, V. S.; Il'inova, M. K. Changes in fatty acid composition and in the content of neutral lipids, glyco- and phospholipids during birch bud and leaf development. *Biol. Nauki (Moscow)* **1988**, *2*, 74−79.

(22) Rodionov, V. S.; Il'inova, M. K.; Shulyakovskaya, T. A. Annual rhythms of lipids concentration and their fatty acids composition in birch buds. *Lesovedenie* **1987**, *4*, 57−64.

(23) Chernobrovkina, N. P.; Il'inova, M. K. Fatty acid composition of glyco- and phospholipids of buds and leaves of the weeping birch. *Lipidnyi Obmen Drev. Rast. Usloviyakh Sev.* **1983**, 112−118.

(24) Ekman, R. Lipophilic extractives of the inner bark of birch, *Betula verrucosa* Ehrh. *Finn. Chem. Lett.* **1983**, *7*−*8*, 162−165.

(25) Hemingway, R. W. Thermal instability of fats relative to surface wettability of yellow birch wood (*Betula lutea*). *Tappi* **1969**, *52* (11), 2149−2155.

(26) Clermont, L. P. The fatty acids of aspen, poplar, basswood, yellow birch, and white birch. *Pulp Paper Mag. Can.* **1961**, *62*, T511−T314.

(27) Aboutabl, E. A.; Nassar, M. I.; Elsakhawy, F. M.; Maklad, Y. A.; Osman, A. F.; El-Khrisy, E. A. M. Phytochemical and pharmacological studies on *Sideritis taurica* Stephan ex Wild. *J. Ethnopharmacol.* **2002**, *82* (2−3), 177−184.

(28) Khattab, A. M.; Nofal, S. M. Chemical and pharmacological investigations of the aerial parts of *Plantago albicans*. *Bull. Fac. Pharm. (Cairo Univ.)* **2001**, *39* (3), 225−234.

(29) Gonzalez Romero, M. A.; Villaescusa C. L.; Diaz Lanza, A. M.; Bartolome, E. C.; Fernandez Matellano, L. Phytochemistry and pharmacological studies of *Inula montana* L. *Recent Res. Dev. Phytochem.* **2001**, *5*, 255−268.

(30) Alagawadi, K. R.; Ronad, P. M.; Mahajanshetti, C. S.; Patil, K. B. Characterization and pharmacological screening of three minor seed oils. *J. Oil Technol. Assoc. India (Mumbai, India)* **2001**, *33* (2), 43−44.

(31) Yff, B. T. S.; Lindsey, K. L.; Taylor, M. B.; Erasmus, D. G.; Jager, A. K. The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. *J. Ethnopharmacol.* **2002**, *79* (1), 101−107.

(32) Sarg, T.; Ateya, A.-M.; Abdel-Ghani, A.; Badr, W.; Shams, G. Phytochemical and pharmacological studies of Dalbergia sissoo growing in Egypt. *Pharm. Biol. (Lisse, Netherlands)* **1999**, *37* (1), 54−62.

(33) Voet, D.; Voet, J. G. *Biochemistry*, 2nd ed.; Wiley: New York, 1995; pp 629.

(34) Tewtrakul, S.; Miyashiro, H.; Nakamura, N.; Hattori, M.; Kawahata, T.; Otake, T.; Yoshinaga, T.; Fujiwara, T.; Supavita, T.; Yuenyongsawad, S.; Rattanasuwon, P.; Dej-Adisai, S. HIV-1 integrase inhibitory substances from *Coleus parvifolius*. *Phytother. Res.* **2003**, *17* (3), 232−239.

(35) Couladis, M.; Chinou, I. B.; Tzakou, O.; Loukis, A. Composition and antimicrobial activity of the essential oil of *Ballota pseudodictamnus* L. Bentham. *Phytother. Res.* **2002**, *16* (8), 723−726.

(36) Saludes, J. P.; Garson, M. J.; Franzblau, S. G.; Aguinaldo, A. M. Antitubercular constituents from the hexane fraction of *Morinda citrifolia* Linn. (Rubiaceae). *Phytother. Res.* **2002**, *16* (7), 683−685.

(37) Chernomorsky, S.; Segelman, A.; Poretz, R. D. Effect of dietary chlorophyll derivatives on mutagenesis and tumor cell growth. *Teratogen., Carcinogen., Mutagen.* **1999**, *19* (5), 313−322.

(38) Simola, L. K.; Koskimies-Soininen, K. Comparison of glycolipids and plastids in callus cells and leaves of *Alnus* and *Betula*. *Plant Cell Physiol.* **1984**, *5* (8), 1329−1340.

(39) Sameshima, K.; Shigematsu, A.; Takamura, N. Biologically active substances in pulping waste liquors. III. Determination of simple monomers in pine and birch kraft pulp waste liquors and their toxicity to fish. *Mokuzai Gakkaishi* **1986**, *32* (5), 344−350.

(40) Yuan, H.; Shi, Y.; Qu, X.; Yao, Q.; Wei, X. Studies on pharmacology and chemical constituents of *Plocamium telfairiae* HARV. *Zhongguo Haiyang Yaowu* **2000**, *19* (5), 7−11.

(41) Ohara, S.; Yatagai, M.; Hayashi, Y. Extractives from the bark of *Betula platyphylla* Sukatchev var. Japonica Hara. *Mokuzai Gakkaishi* **1986**, *32* (4), 266−273.

(42) Dodd, R. S.; Fromard, F.; Rafii, Z. A.; Blasco, F. Biodiversity among West African *Rhizophora*: foliar wax chemistry. *Biochem. Syst. Ecol.* **1995**, *23* (7/8), 859−868.

(43) Fujita, S.; Kawai, K.; Nogami, K. Miscellaneous contributions to the essential oils of plants from various territories. Part III. On the components of the essential oils of myrtle (*Myrtus communis* Linn.). *Mukogawa Joshi Daigaku Kiyo, Kaseigakubuhen* **1992**, *40*, 33−37.

(44) El Alfy, T. S.; Tadros, S. H.; Ibrahim, T. A.; Hassan, A. B.; Elhakim, G. Abd. Phytochemical investigation of *Lagonychium farctum* Banks and Sol. growing in Egypt. *Bull. Fac. Pharm. (Cairo Univ.)* **2000**, *38* (3), 119−128.

(45) Ibrahim, N. A.; El-Gengaihi, S.; El-Hamidi, A.; Bashandy, S. A. E. Chemical and biological evaluation of *Tamarindus indica* L. growing in Sudan (International Symposium on Medicinal and Aromatic Plants). *Acta Hortic.* **1994**, *390*, 51−57.

(46) Ibrahim, N.; Saeed, A.; Bashandy, S.; Omer, E. Mucilage and lipid constituents of *Balanites aegyptiaca* Del. and their biological evaluation. *Bull. Fac. Pharm. (Cairo Univ.)* **1994**, *32* (3), 411−414.

(47) Verardo, G.; Pagani, E.; Geatti, P.; Martinuzzi, P. A thorough study of the surface wax of apple fruits. *Anal. Bioanal. Chem.* **2003**, *376* (5), 659−667.

(48) Fokialakis, N.; Melliou, E.; Magiatis, P.; Harvala, C.; Mitaku, S. Composition of the steam volatiles of six *Euphorbia spp*. from Greece. *Flavour Fragrance J.* **2003**, *18* (1), 39−42.

(49) Perez-Camino, M. C.; Moreda, W.; Mateos, R.; Cert, A. Simultaneous determination of long-chain aliphatic aldehydes and waxes in olive oils. *J. Chromatogr., A* **2003**, *983* (1−2), 283−288.

(50) Guillen, M. D.; Manzanos, M. J. Composition of the extract in dichloromethane of the aerial parts of a Spanish wild growing plant *Thymus vulgaris* L. *Flavour Fragrance J.* **1998**, *13* (4), 259−262.

(51) Guelz, P. G.; Mueller, E. Seasonal variation in the composition of epicuticular waxes of *Quercus robur* leaves. *J. Biosci.* **1992**, *47*, 11−12.

(52) Greenaway, W.; May, J.; Scaysbrook, T.; Whatley, F. R. Compositions of bud and leaf exudates of some *Populus* species compared. *Z. Naturforsch., C: J. Biosci.* **1992**, *47* (5−6), 329−334.

(53) Balsdon, J. A.; Espelie, K. E.; Braman, S. K. Epicuticular lipids from azalea (Rhododendron) and their potential role in host plant acceptance by azalea lace bug, *Stephanitis pyrioides*. *Biochem. Syst. Ecol.* **1995**, *23* (5), 477−485.

(54) Yang, G.; Espelie, K. E.; Todd, J. W.; Culbreath, A. K.; Pittman, R. N.; Demski, J. W. Cuticular lipids from wild and cultivated peanuts and the relative resistance of these peanut species to fall armyworm and trips. *J. Agric. Food Chem.* **1993**, *41*, 814−818.

(55) Ansari, S.; Jain, P.; Tyagi, R. P.; Joshi, B. C.; Barar, F. S. K. Phytochemical and pharmacological studies of the aerial parts of *Eupatorium adenophorum* L. *Herba Pol.* **1983**, *29* (2), 93−96.

(56) Di Biase, A.; Avellino, C.; Pieroni, F.; Quaresima, T.; Grisolia, A.; Cappa, M.; Salvati, S. Effects of exogenous hexacosanoic acid on biochemical myelin composition in weaning and post-weaning rats. *Neurochem. Res.* **1997**, *22* (3), 327−331.

(57) Rowe, J. W. *Natural Products of Woody Plants II*; Springer-Verlag: Berlin, Germany, 1991; pp 771, 1087.

(58) Stevanovic Janezic, T.; Lavoie, J.-M.; Pichette, A.; Gagnon, H.; Jean, F.-I. Squalene: the major constituent of the extracts from yellow birch wood Betula alleghaniensis. Presented at the 10th European Symposium on Phytochemicals and Human Health, Salamanca, Spain, April 18−20, 2002.

(59) Reunanen, M.; Ekman, R.; Hafizoglu, H. Composition of tars from softwoods and birch. *Holzforschung* **1996**, *50* (2), 118−120.

(60) Lindgren, B. O.; Svahn, C. M. Extractives of linden wood (*Tilia vulgaris*). *Phytochemistry* **1968**, *7* (4), 669.

(61) Martinez Moreno, J. M. Substances of dietetic and pharmacological interest found in natural olive oil. *Anstrichmittel* **1964**, *66* (11), 903−907.

(62) Hanna, A. G.; Yassin, F. Y. S.; Allam, R. I.; Yassin, N.; El-Kassaby, I. A phytochemical screening of some solanaceous plants. Phytosterol and fatty acid constituents of *Solanum elaeagnifolium* and *Solanum nigrum* in addition to preliminary pharmacological and antimicrobial investigation. *Egypt. J. Pharm. Sci.* **1996**, *37* (1−6), 211−231.

(63) Jain, R.; Saxena, U. Aliphatics and triterpenoids from the heartwood of *Dichrostachys cinerea*. *J. Indian Chem. Soc.* **2003**, *80* (6), 656−658.

(64) Chen, C.; Chen, Z. Analysis of the *Eucalyptus urophylla* extractives. *Emerging Technologies of Pulping and Papermaking of Fast-Growing Wood*; Proceedings of the International Symposium, Guangzhou, China; Nov 23−25, 1998; pp 103−108.

(65) Khan, M. R.; Mlungwana, S. M. γ-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. *Fitoterapia* **1999**, *70* (1), 96−97.

(66) Estrada, H.; Walls, F.; Santos, E.; Garcia, F.; Flores, S. E. Triterpenes from *Stemmadenia donnell-smithii*. *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.* **1962**, *14*, 19−31.

(67) Kutney, J. P.; Pritchard, P. H.; Ding, Y.; Wasan, K. M. (Forbes Medi-Tech Inc., Can.) Derivatives comprising sterols and/or stanols and specific classes of anti-inflammatory agents and use thereof in treating or preventing cardiovascular disease. PCT Int. Appl. 2004; 75 pp.

(68) Hooper, S. N.; Chandler, R. F. Herbal remedies of the Maritime [Canada] Indians: phytosterols and triterpenes of 67 plants. *J. Ethnopharmacol.* **1984**, *10* (2), 181−194.

(69) Yang, G.; Wiseman, B. R.; Isenhour, D. J.; Espelie, K. E. Chemical and ultrastructural analysis of corn cuticular lipids and their effect on feeding by fall armyworm larvae. *J. Chem. Ecol.* **1993**, *19* (9), 2055−2074.

(70) El Deeb, K. S.; Al-Haidari, R. A.; Mossa, J. S.; Ateya, A. Phytochemical and pharmacological studies of *Maytenus forsskaoliana*. *Saudi Pharm. J.* **2003**, *11* (4), 184−191.

(71) Goswami, U.; Fernandes, N. Biologically active chloroform fraction of an extract obtained from a mangrove plant *Salvadora persica*. U.S. Patent Appl. Publ. 2003; 14 pp.

(72) Meyre-Silva, C.; More, T. C.; Biavatti, M. W.; Santos, A. R. S.; Dal-Magro, J.; Yunes, R. A.; Cechinel-Filho, V. Preliminary phytochemical and pharmacological studies of *Aleurites moluccana* leaves. *Phytomedicine* **1998**, *5* (2), 109−113.

(73) Harraz, F. M.; Ayad, A. R. Phytochemical and biological investigation of *Cleome amblyocarpa* Barr. et Murb. *Zagazig J. Pharm. Sci.* **1994**, *3* (3A), 64−71.

(74) Kweifio-Okai, G.; Macrides, T. A. Antilipoxygenase activity of amyrin triterpenes. *Res. Commun. Chem. Pathol. Pharmacol.* **1992**, *78* (3), 367−372.

(75) Kweifio-Okai, G.; De Munk, F.; Rumble, B. A.; Macrides, T. A.; Cropley, M. Antiarthritic mechanisms of amyrin triterpenes. *Res. Commun. Mol. Pathol. Pharmacol.* **1994**, *85* (1), 45−55.
(76) De Miranda, A. L. P.; Silva, J. R. A.; Rezende, C. M.; Neves, J. S.; Parrini, S. C.; Pinheiro, M. L. B.; Cordeiro, M. C.; Tamborini E.; Pinto, A. C. Anti-inflammatory and analgesic activities of the latex containing triterpenes from *Himatanthus sucuuba*. *Planta Med.* **2000**, *66* (3), 284−286.
(77) Hua, Y.; Bentley, M. D.; Cole, B. J. W.; Murray, K. D.; Alford, A. R. Triterpenes from the outer bark of *Betula nigra*. *J. Wood Chem. Technol.* **1991**, *11* (4), 503−516.
(78) Rimpler, H.; Kuhn, H.; Leuckert, C. Triterpenes of *Betula pendula* and *Betula pubescens*. Comparative study of the bark. *Arch. Pharm.* **1966**, *299* (5), 422−428.
(79) Monaco, P.; Previtera, L. Isoprenoids from the leaves of *Quercus suber*. *J. Nat. Prod.* **1984**, *47* (4), 673−676.
(80) Brevedan, M. I. V.; Carelli, A. A.; Crapiste, G. H. Changes in composition and quality of sunflower oils during extraction and degumming. *Grasas Aceites (Sevilla)* **2000**, *51* (6), 417−423.
(81) Dailey, O. D.; Severson, R. F.; Arrendale, R. F. Nonpolar lipids of *Amaranthus palmeri* S. Wats. 1. Fatty alcohols and wax esters (saturated). *J. Agric. Food Chem.* **1989**, *37*, 1317−1321.
(82) Ilyas, M.; Verma, R.; Jamal, P. Chemical investigation of the leaves of *Breynia rhamnoids* Muell (Euphorbiaceae). *J. Indian Chem. Soc.* **1978**, *155* (9), 964.
(83) Griffiths, D. W.; Robertson, G. W.; Shepherd, T.; Birch, A. N. E.; Gordon, S.; Woodford, J. A. T. A comparison of the composition of epicuticular wax from red raspberry (*Rubus idaeus* L.) and hawthorn (*Crataegus monogyna* Jacq.) flowers. *Phytochemistry* **2000**, *55* (2), 111−116.
(84) Tava, A. Chemical composition of long chain esters in olive fruit (*Olea europaea*). *Ind. Aliment. (Pinerolo, Italy)* **1998**, *37* (366), 28−32.
(85) Arrendale, R. F.; Severson, R. F.; Chortyk, O. T.; Stephenson, M. G. Isolation and identification of the wax esters from the cuticular waxes of green tobacco leaf. *Beitr. Tabakforsch. Int.* **1988**, *14* (2), 67−84.
(86) Hunt, G. M.; Holloway, P. J.; Baker, E. A. Ultrastructure and chemistry of *Clarkia elegans* leaf wax: a comparative study with *Brassica* leaf waxes. *Plant Sci. Lett.* **1976**, *6* (6), 353−360.
(87) Tunalier, Z.; Kosar, M.; Ozturk, N.; Baser, K. H. C.; Duman, H.; Kirimer, N. Antioxidant properties and phenolic composition of *Sideritis* species. *Chem. Nat. Prod.* **2004**, *40* (3), 206−210.

Received for review February 9, 2005. Accepted April 19, 2005. We thank NSERC for a research grant (T.S.) and NSERC and Forintek Canada Corp. for a scholarship (J.-M.L.).

JF050301Y