Chemical Composition of the Unexplored Volatile Fraction of *Betula glandulosa*, a Prevalent Shrub in Nunavik, Québec

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The volatile fraction of the leaves of *Betula glandulosa* MICHX. has been investigated for its secondary metabolite composition by GC-MS and GC/FID. The rapid expansion of this shrub species in subarctic landscapes, like the ones found in Nunavik (Northern Québec, Canada), highly impacts ecosystem dynamics. Yet, despite its abundance, few phytochemical investigations have yet been conducted on this species. In this study, we present the first phytochemical investigation of the volatile metabolites of *B. glandulosa* leaves. Although no essential oil was isolated, volatile compounds were extracted from the hydrosol by steam distillation. The main metabolites observed were linalool (14.6 – 19.0%), C_6 oxylipins (known as green leaf volatiles, GLV; total of 18.2 – 40.2%), eugenol (1.6 – 8.6%) and α-terpineol (3.3 – 4.8%). Dwarf birch is an important food source for insects and herbivores, so knowledge of its metabolite composition could help understand parts of its functional role in subarctic ecosystems. The composition of the volatile fraction could serve as marker for differentiating *B. glandulosa* from other dwarf birch species like *Betula nana* L.

Keywords: Betula glandulosa • Volatile secondary metabolites • Terpenes • Green leaf volatiles • Northern ecosystems

Introduction

Betula glandulosa MICHX. is a shrub species belonging to the *Betula* genus (>100 species) of the Betulaceae family.[1] It is found in Alaska, across Northern Canada, around Hudson Bay, in Labrador, in the maritime provinces of Canada, and in Greenland. Its southernmost distribution reaches into the northern part of the United States, and it can also be found at higher altitudes in continental North America.[2] It stands 0.3–2.5 m tall and can develop either an erect or prostrate growth form. Its branches have dark brown bark which does not exfoliate. Its leaves are ovate to orbicular, rounded at the apex and the base with less than 10 crenations on each edge. Leaves are usually 0.5–2 cm long and 0.5–1.5 cm wide. They present a dark green color above and a yellow green to dark green color below.[2] The overall morphology of the leaves and of the shrub as well as an example of the environment where it can be found are shown in Figure 1. Another dwarf birch, *Betula nana* L., is morphologically very similar to *B. glandulosa*, making those two species difficult to differentiate in regions where they are both found.[2] *Betula glandulosa* is consumed and used by Dene and Chipewyan peoples, First Nations inhabiting the Canadian boreal forest. The whole plant has traditionally been used to prepare tea for the treatment of stomach problems, leaves are used to treat insect bites, and chewed twigs can be applied on deep cuts.[3] Other birch species have traditionally been used for medicinal purposes by different communities in the Canadian boreal forest[3] and in other regions of the world[4].

The ongoing expansion of shrub species in northern regions, referred to as shrubification from here on, has been linked to a variety of drivers : warmer temperatures, deeper snow cover, permafrost thaw, tundra fires, and biotic disturbances associated with herbivores and humans.[5] Shrubification alters the competitive environment in the subarctic terrestrial ecosystems and is detrimental to lower vascular plants and lichens, which are not able to compete with erect shrubs.[5] Erect shrub structure influences the snow cover[6, 7], the soil dryness when snow melts[7, 8], the carbon and energy exchanges between ecosystems and the atmosphere, and the atmospheric heat flux[9-12]. Depending on the total balance of these impacts, global warming could be accelerated by factors influenced by the greening of northern ecosystems.[13] *Betula glandulosa* has been one of the species associated with the shrubification of northern regions[14] as observed in Eastern Canada, and particularly in Nunavik (Québec, Canada) near the tree line[15-17].

Despite its abundance in Nunavik and other regions of Northern Canada, *B. glandulosa* has not received much attention from phytochemists. As a result, its secondary metabolites remain almost unstudied. The only published investigation of *B. glandulosa*'s secondary metabolism led to the identification of three triterpenes (including the previously unknown deacetoxypapiriferic acid, dammar-24-ene-12-β-*O*-acetyl-2-*O*-(*S*)-ol-3-one, which was previously known only from synthesis, and papyriferic acid) in a fraction considered to be responsible for the deterrent properties of this shrub against browsing by snowshoe hares.[18] Previous work on non-volatile secondary metabolites in our lab indicated that *B. glandulosa's* leaves could contain some interesting

volatile metabolites, based on the fragrance that has been perceived. It has also been demonstrated that this species produces defensive secondary metabolites, which could be linked to low insect herbivory.[19]

Figure 1. *Betula glandulosa* leaves (a) and stems (b). Harvesting was conducted in a lichen woodland (c).

Even though no report on *Betula glandulosa*'s volatile metabolites has been published, other birch species have been phytochemically investigated for their volatile compounds, including other shrub species of the *Betula* genus. The essential oils of leaves from birch species are commonly dominated by sesquiterpenoids of the caryophyllene type like α-betulenol, 14-hydroxy-4,5-dihydro-β-caryophyllene, and α-betulenol acetate.[20, 21] Other sesquiterpenoids have been identified as the main compounds in some extracts.[20-24] Some birch species have been found to yield essential oils containing other types of secondary metabolites as their main components. The leaves from *B. nana* were dominated by aliphatic compounds (*n*-tricosane, *n*-pentacosane, *n*-eicosane, *n*-heneicosane, *n*-nonadecane) instead of sesquiterpenoids like other birch species in the same study.[21] The leaves from *B. medwediewii* were quite different from the other species in this study, containing mostly methyl salicylate.[21] Essential oil extracted from the leaves of *B. nigra* contained mainly (*E*)-2-hexenal, linalool, and eugenol, and no sesquiterpenoids were observed.[25] Essential oils and volatile compounds from birch buds and branches have also been studied. In many cases, the secondary metabolite composition of the extracts was similar to those obtained from the leaves, with sesquiterpenoids as the main components.[20, 26] In some other cases, buds were found to have a very different composition, such as with *B. medwediewii* buds, which contained mostly α-betulenol and 14-hydroxy-4,5-dihydro-β-caryophyllene, while methyl salicylate dominated the essential oil in *B. medwediewii* leaves.[21] Fatty acid derivatives, including C6 oxylipins referred to as green leaf volatiles (GLVs; for example: hexanal, hexanol and hexenal), were significant in the composition of volatile emissions, volatile extracts and essential oils from birch species.[21, 22, 25, 27]

In several studies on volatile secondary metabolites from birch, variability within a species has been linked to stresses or biological factors, as observed for other plant species. Biotic factors like physical perturbations (parasites, pathogens or herbivores attacks)[28-30], genetics[23], maturity[30] and phenology[30, 31] have been proven to influence the content of some volatile secondary metabolites like GLVs and terpenoids. Some studies have reported that abiotic factors of drought[22], CO_2 levels[28], light[30] and temperature[30, 32-34] can affect birches' production of certain volatile secondary metabolites.

In this study, we first evaluated the potential of *B. glandulosa*'s leaves from the Whapmagoostui-Kuujjuarapik region, in Nunavik, Québec, to produce essential oil. Subsequently, we characterized its volatile secondary metabolite content to document the phytochemistry of this abundant shrub from northern regions. This information could be useful in chemotaxonomy and provide useful insights on the defensive metabolites produced by this phytochemically understudied birch species.

Results and Discussion

Steam distillation and extraction processes were applied to two different leaf samples (Sample A and Sample B). We intended to use steam distillation to produce essential oil from *B. glandulosa's* freshly harvested leaves from the Whapmagoostui-Kuujjuarapik region in Nunavik, Québec, Canada. During the distillation process, a thin film formed on the surface of the aqueous phase in the separator for both leaf samples, with a whitish color and a viscous and foamy appearance. Although it did not look like an oil, it was floating on the hydrosol. After the distillation process, it was retrieved along with some hydrosol in a vial, where it eventually mixed with the aqueous phase. The very small volume obtained and its apparent miscibility with water made it very difficult to decant or retrieve it without hydrosol. Therefore, it was extracted with diethyl ether, along with the rest of the hydrosol retrieved from the separator of the steam distillation apparatus. The two samples presented hereby are volatile extracts obtained by liquid-liquid extraction of the hydrosol after a steam distillation with a Clevenger type apparatus with cohobation.

The samples obtained following liquid-liquid extraction of the hydrosol and evaporation were a very pale yellowish viscous oil with a strong, fruity, woody smell. The amounts obtained were low (38 and 46 mg for Samples A and B, respectively) since the extraction was done only on the hydrosol collected in the separator (around 225 mL per sample), following a steam distillation with cohobation. The yield could probably have been improved by focusing on

extracting a volatile fraction instead of an essential oil, from the start. Methods such as simultaneous distillation-extraction (SDE)[35], steam distillation with a solvent trap in the Clevenger apparatus, or solvent extraction might have provided a greater amount of volatile compounds.

The GC-MS analyses allowed the identification of around 90% of the extracts (in relative proportions), corresponding to 119 and 142 secondary metabolites for Samples A and B, respectively. The list of compounds identified in this study is presented in Table 1, along with their identification information and relative proportions. Their GC-MS chromatograms are presented in Figure 2, for a visual comparison of the samples' composition. The relative abundance of the main compounds identified in the samples differs. Sample A was dominated by linalool (19.0%) followed by eugenol (8.6%) and some fatty acid derivatives described as GLVs[27] (18.2% in total). Sample B contained mainly GLVs (40.2% in total including 23.2% of (*Z*)-3-hexen-1-ol), followed by linalool (14.6%); the eugenol content (1.6%) was much lower. Chemical structures of the main compounds (accounting for at least 2% of relative proportion) are presented in Figure 3. The identification is based on mass spectra and the linear retention index on non-polar column. A polar stationary phase column was used to confirm most of the identifications with a second linear retention index. The absence of some retention index on polar column in databases, the small relative proportion of some compounds with mass spectrometry detection, and the possible lack of resolution of some peaks did not allow for the confirmation of all the identified compounds with a linear retention index on a second column polarity. The list of unidentified compounds representing more than 0.1% of the volatile extract is available in the supplementary information with mass spectrometry and gas chromatography data.

Our results show the presence of secondary metabolites produced via different biosynthesis pathways. Both extracts contained compounds belonging to the terpene class, the phenylpropanoid class, compounds derived from benzoic acid, and compounds derived from fatty acids. The amount of each compound in each class are presented in Table 1, allowing a comparison of the two samples. Even though they were located close to each other (ca. 6 km), the two harvest sites near the Whapmagoostui-Kuujjuarapik communities presented structural differences that could lead to significant variations of the chemical composition of the *B. glandulosa* individuals. Tree and shrub cover at Site A was denser than at Site B. These structural differences in vegetation cover could explain a part of the variability of the volatile secondary metabolites' composition of *Betula glandulosa*'s leaves, although this interpretation is only based on two sites that do not necessarily reflect the natural variability encountered in the region.

It is known from previous reports that the composition of volatile secondary compounds can vary widely depending on a variety of factors. GLVs are recognized as signaling metabolites.[34] They are C_6 compounds and their acetates, they are derived from fatty acids, and they are formed via the lipoxygenase pathway.[22] They can be present in plants as the result of biotic disturbances (*i.e.* wounds caused by herbivores, insects or pathogens as reported for other plant species).[36-38] Theses volatile compounds have a repellent effect on certain insects, which could be a reason for their biosynthesis by organisms.[31] They seem to play key roles in plant communications with their surroundings.[39-42] GLV concentration can be influenced by manipulations during harvesting. For example, the removal of twigs has led to a substantial rise, up to 20%, in GLV concentration for *B. pendula*[28], while the pressing of leaves and twigs on living *B. pubescens* induced important emissions of (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, 2-hexenal and 1-hexanol, even though this perturbation had only a short-term impact (*ca.* 1 hour) on these emissions[30]. Drought stress had a positive impact on GLV emission levels by *B. verrucosa*.[22] Moreover, monoterpenoid concentration in emissions, especially linalool emissions, could be associated with the time of harvest; this was shown for *B. pubescens*, which produces higher amounts of linalool at the beginning of the growing season, and less in the second part of the growth season.[30] Temperature also has an impact on linalool emissions by *B. pendula* and *B. pubescens*.[30]

In our study, we observed differences between the two *B. glandulosa* samples, even though the harvesting, sample treatment and extraction were identical for both samples. Samples were harvested within two days in the same region, although they were harvested on two different days and at different time, with slight differences of temperature and meteorological factors. Both days were sunny, but sample A was harvested in the late afternoon at around 12 °C and sample B was harvested in the morning at 8 – 10 °C. Yet, differences between the samples were important for the monoterpenoids (40.9% and 20.5% respectively) and were inversely proportional to GLVs (20.3% and 42.3% respectively). Without a more robust quantitative analysis, these results are hard to evaluate, the proportions for monoterpenoids could be biased by the level of measured GLVs, and these last compounds are highly variable in response to external factors, as mentioned previously. The relative proportions of phenylpropanoids and benzoic acid-type metabolites was different between the samples (16.3% and 5.0% for Samples A and B), with eugenol being the main compound from these classes (8.6% and 1.6%). Again, as for monoterpenoids, these relative proportions could be biased by the differences in the amounts of GLVs. The production of phenylpropanoids and benzoic acid derivatives have not been linked to specific factors in birches. The influence of several factors on the phenylpropanoid content has been particularly studied in species of the *Ocimum* genus and soil salinity[43], temperature[44, 45], solar irradiance[46], leaf development[47] and genotype[48] all have an impact on phenylpropanoid levels, including eugenol. Therefore, our results emphasize the need for further studies to better understand the volatile secondary metabolite biosynthesis by this shrub species.

Even though differences have been observed between the two samples, they also shared some common features; most of the compounds were found in both samples. Linalool and GLVs were major compounds of both samples. *B. glandulosa* from the Whapmagoostui-Kuujjuarapik region seems to produce mostly the same volatile secondary metabolites, but in different proportions. Although our results were obtained with a limited number of specimens, it appears that the common secondary metabolites could be characteristic of the species.

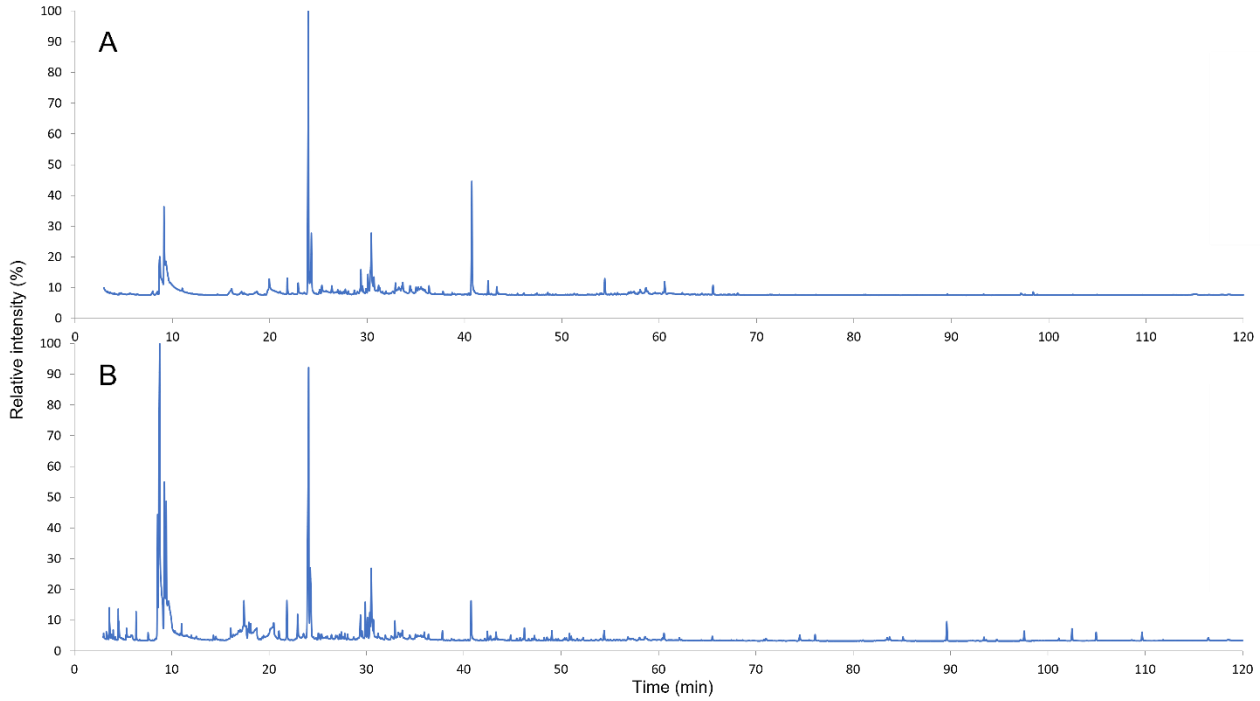

Figure 2. GC-MS chromatograms of *Betula glandulosa* leaves' volatile fractions. A) chromatogram for volatile extract of sample A, B) chromatogram for volatile extract of sample B. 1: (*E*)-2-hexenal; 2: 3-(*Z*)-hexen-1-ol; 3: 2-(*E*)-hexenol; 4: hexanol; 5: benzyl alcohol; 6: linalool; 7: phenethyl alcohol; 8: α-terpineol; 9: eugenol.

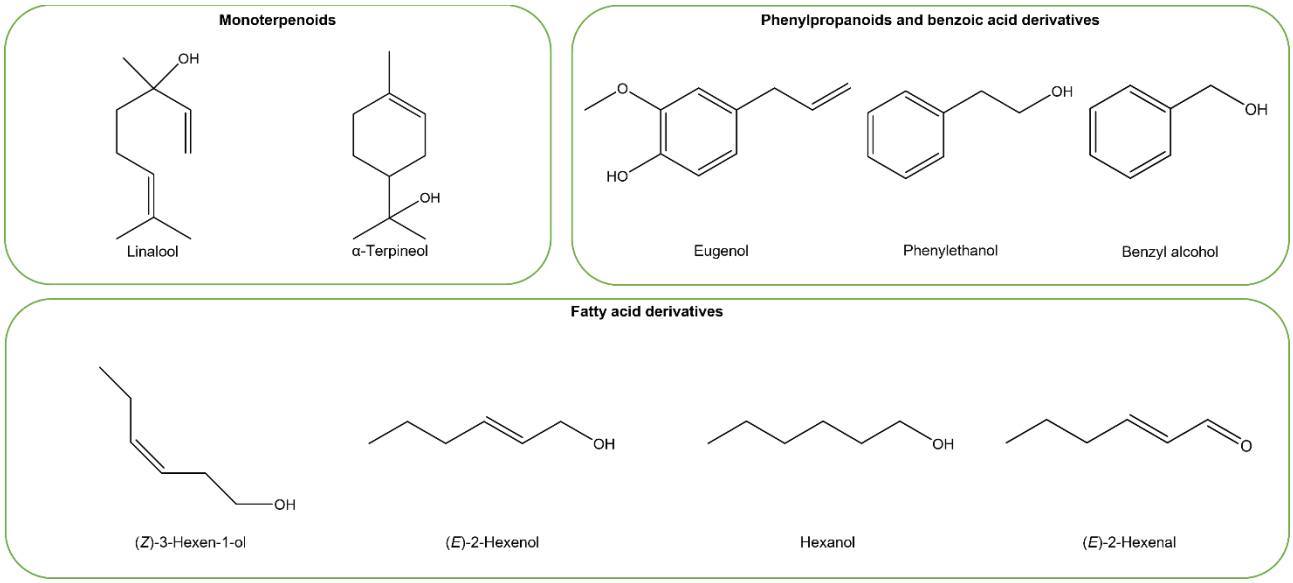

Figure 3. Main compounds observed in *Betula glandulosa*'s leaves volatile fraction.

Investigations on volatile birch compounds has shown that some secondary metabolites could be indicative of the species.[23, 49] By comparing data available in the literature for other birch species, the volatile compounds observed in *B. glandulosa* show some particularities that could distinguish it from others. The prevalence of linalool is not unique to *B. glandulosa* as other members of the *Betula* genus such as *B. nigra*[25] and *B. utilis*[50] have shown high amounts of the monoterpenoid in their essential oil or volatile emissions. For many species, like *B. pendula*[20, 21, 23], *B. pubescens*[20, 23], *B. browicziana*[21], *B. litwinowii*[21], *B. recurvata*[21] and *B. humilis*[20], observed monoterpenoids are less important and their essential oils or volatile emissions are dominated by sesquiterpenes and sesquiterpenoids, which are classes of compounds that are present in *B. glandulosa*, but in smaller amounts. Phenylpropanoids, such as eugenol, have been identified in some birch species. *Betula nigra*'s leaf essential oil contains high amount of eugenol.[25] This volatile compound has also been identified in *B. pendula*, *B. browicziana*, *B. litwinowii*, *B. medwediewii* and *B. recurvata* essential oils in proportions up to 1.7%,[21] and in *B. pendula*, *B. pubescens* and their hybrids in proportions up to 4.08% (observed for *B. pendula*)[23]. Interestingly, in a study comparing volatile compounds in different birch species, *B. medwediewii* yielded very different essential oils from its branches and leaves, both being largely dominated by methyl salicylate, a benzoic acid derivative.[21] To fully assess the possibility of using volatile compounds as chemical markers for chemotaxonomy, more studies should focus on the

phytochemistry of birch species and on the comparison of the content in volatile secondary metabolites in the different species. Such comparisons have been reported previously, for example, terpenoids in the leaves and the buds could be useful as markers for hybrid recognition.[23] It has been proposed that seasonal variation is less important than interspecific variation.[51] Studies on buds also demonstrated great specificity for birch species' metabolites.[49]

In the case of *B. glandulosa*, it would be particularly useful to be able to distinguish it from *B. nana*, another birch shrub which is morphologically very similar. The two species can both be found in specific areas, along with hybrid species.[2] *Betula nana* has been the subject of some phytochemical studies, but data are still limited. Its leaf essential oil (obtained as traces) was very different from the other birch species in a study in Estonia, containing mainly aliphatic compounds and only very little sesquiterpenoids.[20] Compared to our results for *B. glandulosa*'s volatile extracts, *B. nana*'s essential oil contained only a small proportion of linalool, and eugenol was not observed.[20] Linalool was not observed either in the biogenic volatile organic compound emissions of *B. nana* from Greenland.[52] Further studies on both these species will provide phytochemical information that could indicate if these differences can be linked to genetics instead of other factors. The procedure followed for harvesting the samples, treating the biomass, preparing the extracts and analyzing the samples should be well controlled or, at least, taken into consideration. The comparisons made with literature data in this article imply different procedures and type of extracts and are to be considered with care.

In addition to the chemotaxonomic role that this study suggests, it is interesting to gather phytochemical information on *B. glandulosa*.[5, 15-17] This species grows in northern regions of North America where climatic changes are already having a serious influence on organisms.[53] Stresses associated with the habitats of *B. glandulosa* are varied as it is distributed over large areas, encompassing different vegetation zones[2]. As climate change will disturb these areas and force organisms to adapt to new conditions, it could lead to changes in their secondary metabolite production to better protect themselves. Some phytochemical information could be lost or, at least, altered by these adaptations.

Table 1. Composition of the volatile fraction obtained from *Betula glandulosa* leaves from Whapmagoostui-Kuujjuarapik region.

Compounds	LRI (non-polar column) [a]		LRI (polar column) [b]		Relative proportion [c] [% ± SD]		Identification
	exp.	lit.	exp.	lit.	A	B	
1-Penten-3-ol	691	684[f]	1160	1159	0.1 ± 0.0	0.9 ± 0.0	MS, LRI[i,j]
3-Pentanone	699	697[e]	-	-	-	tr	MS, LRI[i]
3-Pentanol	701	710[e]	1113	1110	0.1 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
Acetoin	706	713[g]	-	-	tr	0.2 ± 0.0	MS, LRI[i]
Isopentyl alcohol	728	729[e]	1210	1209	0.1 ± 0.0	0.6 ± 0.0	MS, LRI[i,j]
2-Methyl-1-butanol	731	731[e]	1210	1208	0.1 ± 0.0	0.4 ± 0.0	MS, LRI[i,j]
Tiglic aldehyde	744	745[g]	-	-	0.1 ± 0.0	tr	MS, LRI[i]
(*Z*)-2-Pentenol	759	765[f]	1325	1318	0.1 ± 0.0	0.4 ± 0.0	MS, LRI[i,j]
3-Methyl-2-butenal	778	778[f]	-	-	tr	tr	MS, LRI[i]
Isobutenyl methyl ketone	798	797[e]	-	-	-	tr	MS, LRI[i]
Hexanal	801	801[e]	1086	1083	0.1 ± 0.0	0.6 ± 0.0	MS, LRI[i,j]
Furfural	824	828[f]	-	-	0.5 ± 0.0	0.3 ± 0.0	MS, LRI[i]
(*E*)-3-Hexen-1-ol	843	844[f]	1367	1367	tr	tr	MS, LRI[i,j]
(*E*)-2-Hexenal	849	850[e]	1220	1216	0.3 ± 0.0	4.1 ± 0.1	MS, LRI[i,j]
(*Z*)-3-Hexen-1-ol	857	853[e]	1390	1382	6.5 ± 0.1	23.2 ± 0.1	MS, LRI[i,j]
(*E*)-2-Hexenol	869	864[e]	1412	1405	6.2 ± 0.3	7.2 ± 0.2	MS, LRI[i,j]
Hexanol	874	868[g]	1357	1355	5.1 ± 0.2	5.0 ± 0.2	MS, LRI[i,j]
2-Methylbutanoic acid	875	881[e]	1691	1662	tr	0.3 ± 0.1	MS, LRI[i,j]
2-Heptanone	888	889[f]	-	-	tr	0.1 ± 0.0	MS, LRI[i]
(*Z*)-4-Heptenal	900	902[e]	1241	1240	tr	0.1 ± 0.0	MS, LRI[i,j]
Heptanal	902	901[f]	1188	1184	0.2 ± 0.0	0.3 ± 0.0	MS, LRI[i,j]
2-Acetylfuran	907	909[f]	1505	1499	tr	tr	MS, LRI[i,j]
(*E*,*E*)-2,4-Hexadienal	909	907[f]	1402	1400	tr	0.1 ± 0.0	MS, LRI[i,j]
(*Z*)-3-Hexenyl formate	917	920[e]	-	-	tr	0.1 ± 0.0	MS, LRI[i]
Hexyl formate	925	929[e]	-	-	tr	tr	MS, LRI[i]
(*E*)-3-Hepten-2-one	931	932[e]	-	-	-	tr	MS, LRI[i]
Ethyl tiglate	934	938[e]	-	-	tr	-	MS, LRI[i]
(*E*)-2-Heptenal	952	956[e]	1323	1323	tr	0.2 ± 0.0	MS, LRI[i,j]

Benzaldehyde	955	952[f]	1518	1520	0.1 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
(*Z*)-4-Hepten-1-ol	962	960[e]	-	-	tr	tr	MS, LRI[i]
Heptanol	968	970[e]	-	-	tr	tr	MS, LRI[i]
1-Octen-3-one	973	973[e]	-	-	tr	tr	MS, LRI[i]
1-Octen-3-ol	978	978[e]	1456	1450	0.4 ± 0.1	tr	MS, LRI[i,j]
6-Methyl-5-hepten-2-one	982	981[f]	1338	1338	0.3 ± 0.1	0.3 ± 0.0	MS, LRI[i,j]
6-Methyl-5-hepten-2-ol	992	994[g]	-	-	0.6 ± 0.1	0.1 ± 0.0	MS, LRI[i]
Ethyl hexanoate	999	997[f]	1234	1233	tr	0.1 ± 0.0	MS, LRI[i,j]
Octanal	1003	1006[e]	1290	1289	tr	0.2 ± 0.0	MS, LRI[i,j]
(*Z*)-3-Hexen-1-ol acetate	1005	1004[f]	1319	1315	tr	1.1 ± 0.1	MS, LRI[i,j]
(*E, E*)-2,4-Heptadienal	1009	1013[e]	1490	1495	0.6 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
Hexyl acetate	1012	1012[e]	-	-	0.3 ± 0.0	0.2 ± 0.0	MS, LRI[i]
(*E*)-2-Hexen-1-ol acetate	1014	1017[e]	1336	1333	0.2 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
Benzyl alcohol	1032	1036[g]	1877	1870	2.5 ± 0.0	1.2 ± 0.0	MS, LRI[i,j]
Phenylacetaldehyde	1038	1036[f]	-	-	0.3 ± 0.1	0.1 ± 0.0	MS, LRI[i]
(*E*)-2-Ethyl-hexenoate	1042	1041[e]	704[h]	709[h]	0.2 ± 0.1	tr	MS, LRI[i,j]
o-Cresol	1053	1051[e]	2014	2008	0.7 ± 0.1	-	MS, LRI[i,j]
(*E*)-2-Octen-1-al	1055	1053[e]	1426	1429	0.6 ± 0.0	0.5 ± 0.0	MS, LRI[i,j]
(*Z*)-Linalool oxide (furanoid)	1068	1069[e]	1439	1444	1.0 ± 0.0	1.4 ± 0.0	MS, LRI[i,j]
(*Z*)-5-Octen-1-ol	1072	1073[e]	1621	1615	0.2 ± 0.0	tr	MS, LRI[i,j]
p-Cresol or *m*-cresol	1075	1072[e] or 1073[e]	- -	- -	tr	-	MS, LRI[i]
Fenchone	1082	1083[f]	1387	1401	-	0.1 ± 0.0	MS, LRI[i,j]
(*E*)-Linalool oxide (furanoid)	1084	1084[f]	1466	1452	0.9 ± 0.0	0.9 ± 0.0	MS, LRI[i,j]
3-Decyn-2-ol	1092	1101[g]	-	-	0.2 ± 0.0	0.3 ± 0.0	MS, LRI[i]
Linalool	1104	1101[e]	1555	1547	19.0 ± 0.3	14.6 ± 0.2	MS, LRI[i,j]
(*E*)-6-Methyl-3,5-heptadien-2-one	1104	1102[e]	951	950	tr	tr	MS, LRI[i,j]
Hotrienol	1105	1107[g]	1616	1613	1.4 ± 0.2	2.2 ± 0.3	MS, LRI[i,j]
Nonanal		1104[e]	1393	1391			
Phenylethyl alcohol	1110	1113[e]	1908	1906	3.7 ± 0.1	1.5 ± 0.0	MS, LRI[i,j]
(*E, E*)-2,4-Octadienol	1112	1113[f]	-	-	0.2 ± 0.0	tr	MS, LRI[i]
β-Thujone	1114	1112[f]	1431	1448	-	0.2 ± 0.0	MS, LRI[i,j]
Dehydrosabinaketone	1115	1117[f]	-	-	0.2 ± 0.0	0.2 ± 0.0	MS, LRI[i]
trans-*p*-Mentha-2,8-dien-1-ol	1119	1119[f]	1627	1639	0.6 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
Limona ketone	1126	1131[e]	1544	-	-	tr	MS, LRI[i]
Nopinone	1131	1135[f]	-	-	-	tr	MS, LRI[i]
cis-*p*-Mentha-2,8-dien-1-ol	1133	1133[f]	1670	1660	0.5 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
trans-Pinocarveol	1134	1135[f]	1648	1664	0.1 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
Camphor	1139	1141[f]	1499	1520	0.2 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
(*Z*)-3-Hexenyl isobutyrate	1141	1142[f]	-	-	tr	0.1 ± 0.0	MS, LRI[i]
4-Acetyl-1,4-dimethyl-1-cyclohexene	1144	1149[g]	-	-	tr	0.2 ± 0.0	MS, LRI[i]
2-Ethylhexyl acetate	1148	1149[f]	745[h]	745[h]	tr	tr	MS, LRI[i,j]
Nerol oxide	1148	1152[e]	831[h]	833[h]	0.2 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
(*E,Z*)-2,6-Nonadienal	1149	1150[f]	-	-	0.1 ± 0.0	-	MS, LRI[i]
Sabina ketone	1151	1154[f]	-	-	0.3 ± 0.0	0.1 ± 0.0	MS, LRI[i]
β-Pinene oxide	1153	1154[f]	-	-	tr	0.1 ± 0.0	MS, LRI[i]
Pinocarvone	1155	1160[f]	-	-	0.3 ± 0.0	0.2 ± 0.0	MS, LRI[i]
(*E*)-2-Nonenal	1157	1157[f]	1529	1534	0.3 ± 0.1	tr	MS, LRI[i,j]
Ethyl benzoate	1164	1169[f]	1659	1658	0.3 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
(*Z*)-Linalool oxide (pyranoid)	1167	1169[e]	-	-	tr	0.1 ± 0.0	MS, LRI[i]

(*E*)-Linalool oxide (pyranoid)	1173	1173[f]	1738	1739	0.2 ± 0.0	tr	MS, LRI[i,j]
Terpinen-4-ol	1175	1174[f]	1597	1602	1.9 ± 0.1	0.9 ± 0.0	MS, LRI[i,j]
Thuj-3-en-10-al	1177	1181[f]	-	-	0.5 ± 0.1	0.2 ± 0.0	MS, LRI[i]
trans-*p*-Mentha-1(7),8-dien-2-ol	1185	1187[f]	1794	1811	0.4 ± 0.0	1.3 ± 0.0	MS, LRI[i]
(*Z*)-3-Hexenyl butanoate	1185	1184[f]	1460	1455	1.5 ± 0.1	0.7 ± 0.0	MS, LRI[i,j]
Methyl salicylate		1190[f]	1762	1765			MS, LRI[i,j]
Myrtenal	1188	1193[g]	1612	1647	1.1 ± 0.1	0.8 ± 0.1	MS, LRI[i]
α-Terpineol	1192	1195[e]	1694	1697	4.8 ± 0.1	3.3 ± 0.1	MS, LRI[i,j]
(-)-*trans*-Isopiperitenol	1196	1210[g]	1748	1745	1.0 ± 0.1	0.7 ± 0.0	MS, LRI[i,j]
(*Z*)-Dihydrocarvone	1198	1198[e]	1195	1195	tr	tr	MS, LRI[i,j]
4-Caranone isomer (*cis*- or *trans*-)	1200	1200[f] or 1200[e]	1602	-	0.4 ± 0.1	0.2 ± 0.0	MS, LRI[i]
Decanal	1204	1201[f]	-	-	0.7 ± 0.1	0.1 ± 0.0	MS, LRI[i]
3-Caren-10-al	1207	-	1711	1711	tr	0.1 ± 0.0	MS, LRI[j]
(-)-*cis*-Isopiperitenol	1212	1228[g]	1742	1742	0.5 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
(*E*)-Carveol	1215	1215[f]	1834	1845	0.2 ± 0.1	tr	MS, LRI[i,j]
(-)-Myrtenol	1219	1213[g]	-	-	-	tr	MS, LRI[i]
Nerol	1223	1227[f]	1802	1797	0.3 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
cis-*p*-Mentha-1(7),8-dien-2-ol	1225	1227[f]	1887	1896	0.8 ± 0.1	0.6 ± 0.0	MS, LRI[i,j]
Isogeraniol	1227	1240[g]	1816	1820	0.4 ± 0.0	-	MS, LRI[i,j]
(*Z*)-3-Hexenyl-2-methyl butanoate	1229	1231[e]	837[h]	837[h]	0.6 ± 0.1	0.2 ± 0.0	MS, LRI[i,j]
Hex-(*Z*)-3-enyl-but-(*E*)-2-enoic acid ester	1233	1237[e]	1599	1599	0.4 ± 0.0	0.1 ± 0.0	MS, LRI[i,j]
Cuminaldehyde	1234	1238[f]	1766	1789	0.5 ± 0.0	0.1 ± 0.0	MS, LRI[i]
Carvone	1237	1239[f]	1718	1741	0.9 ± 0.1	0.3 ± 0.0	MS, LRI[i]
Geraniol	1250	1249[f]	1852	1847	1.1 ± 0.1	0.3 ± 0.0	MS, LRI[i,j]
Chavicol	1250	1252[e]	2346	2334	0.3 ± 0.0	-	MS, LRI[i,j]
(*Z*)-2-Decenal	1259	1260[f]	1636	1644	0.2 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
Ethyl salicylate	1260	1266[f]	-	-	tr	0.1 ± 0.0	MS, LRI[i]
Perillaldehyde	1266	1269[f]	-	-	0.3 ± 0.0	tr	MS, LRI[i]
Phellandral	1268	1277[e]	-	-	0.1 ± 0.0	tr	MS, LRI[i]
Bornyl acetate	1277	1284[f]	1570	1581	0.5 ± 0.0	0.2 ± 0.0	MS, LRI[i,j]
p-Cymen-7-ol	1287	1289[f]	2099	2113	tr	tr	MS, LRI[i,j]
Carvacrol	1294	1298[f]	1573[h]	1565[h]	tr	tr	MS, LRI[i,j]
(*E*,*E*)-2,4-Decadienal	1313	1315[f]	1800	1811	tr	tr	MS, LRI[i,j]
3-Hexenyl tiglate isomer ((*E*) or (*Z*))	1319	1319[e] or 1319[f]	- -	- -	0.2 ± 0.0	0.1 ± 0.0	MS, LRI[i] MS, LRI[i]
1,5,5-Trimethyl-6-methylene-cyclohexene	1340	1338[g]	-	-	0.1 ± 0.0	tr	MS, LRI[i]
Eugenol	1345	1356[f]	2164	2169	8.6 ± 0.1	1.6 ± 0.0	MS, LRI[i,j]
α-Ylangene	1365	1371[e]	1477	1491	-	tr	MS, LRI[i,j]
β-Damascenone isomer ((*E*) or (*Z*))	1370	1379[e] or 1361[f]	1807	E : 1823 Z : 1794	0.9 ± 0.0	0.3 ± 0.0	MS, LRI[i,j]
β-Bourbonene	1373	1382[e]	-	-	-	tr	MS, LRI[i]
(*Z*)-Jasmone	1383	1392[f]	1925	1906	0.6 ± 0.0	0.3 ± 0.0	MS, LRI[i,j]
(*Z*)-Caryophyllene	1406	1408[f]	1578	1589	-	0.2 ± 0.0	MS, LRI[i,j]
(*E*)-α-Bergamotene	1425	1432[e]	1575	1580	-	0.1 ± 0.0	MS, LRI[i,j]
Guaia-6,9-diene	1431	1442[f]	-	-	-	0.5 ± 0.0	MS, LRI[i]
Aromadendrene	1437	1438[e]	1624	1618	-	0.1 ± 0.0	MS, LRI[i,j]
α-Humulene	1442	1452[f]	-	-	tr	tr	MS, LRI[i]
trans-β-Ionone	1469	1487[f]	1923	1940	tr	-	MS, LRI[i,j]

β-Chamigrene	1475	1476[f]	1701	1725	-	0.4 ± 0.0	MS, LRI[i]
α-Muurolene	1488	1497[e]	-	-	-	0.1 ± 0.0	MS, LRI[i]
(*Z*)-α-Bisabolene	1500	1503[e]	-	-	-	0.1 ± 0.0	MS, LRI[i]
(*E*)-Calamenene	1510	1521[f]	1814	1826	-	0.2 ± 0.0	MS, LRI[i,j]
1,2,3,4,4a,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	1520	1533[g]	-	-	-	0.1 ± 0.0	MS, LRI[i]
(*E*)-α-Bisabolene	1533	1540[e]	-	-	-	0.1 ± 0.0	MS, LRI[i]
(*E*)-Nerolidol	1555	1561[e]	2044	2042	tr	0.1 ± 0.0	MS, LRI[i,j]
Caryophyllene oxide	1566	1582[f]	1952	1989	1.1 ± 0.0	0.5 ± 0.0	MS, LRI[i]
Isoaromadendrene epoxide	1589	1589[g]	-	-	0.2 ± 0.0	0.1 ± 0.0	MS, LRI[i]
Caryophylla-4(12),8(13)-dien-5α-ol	1622	1637[g]	-	-	0.3 ± 0.0	0.2 ± 0.0	MS, LRI[i]
tau-Cadinol	1632	1638[f]	-	-	0.1 ± 0.0	tr	MS, LRI[i]
Methyl jasmonate	1633	1638[g]	-	-	0.6 ± 0.0	0.1 ± 0.0	MS, LRI[i]
Cubenol	1642	1645[f]	2045	2080	0.9 ± 0.1	0.2 ± 0.0	MS, LRI[i]
14-hydroxy-(*Z*)-Caryophyllene	1656	1664[e]	-	-	0.1 ± 0.0	-	MS, LRI[i]
Germacrone	1675	1693[f]	2199	2217	1.0 ± 0.1	0.4 ± 0.0	MS, LRI[i,j]
14-hydroxy-4,5-dihydro-Caryophyllene	1708	1706[f]	-	-	tr	tr	MS, LRI[i]
Neophytadiene	1837	1836[e]	-	-	-	tr	MS, LRI[i]
Octadecanol	2080	2081[e]	-	-	-	tr	MS, LRI[i]
Phytol	2103	2106[e]	2615	2622	-	0.1 ± 0.0	MS, LRI[i,j]
Tricosane	2299	2300[e]	-	-	0.1 ± 0.0	0.8 ± 0.0	MS, LRI[i]
1-Heneicosanol	2393	2380[g]	-	-	-	0.2 ± 0.0	MS, LRI[i]
Docosanal	2423	2430[g]	-	-	-	0.1 ± 0.0	MS, LRI[i]
Pentacosane	2499	2500[e]	-	-	0.1 ± 0.0	0.4 ± 0.0	MS, LRI[i]
Tetracosanal	2628	2632[g]	-	-	-	0.4 ± 0.3	MS, LRI[i]
Heptacosane	2699	2700[e]	-	-	tr	0.4 ± 0.1	MS, LRI[i]
Hexacosanal	2832	2832[g]	-	-	-	0.3 ± 0.2	MS, LRI[i]
Total terpenoids {68}[d]					44.6 ± 1.6 {51}[d]	33.0 ± 0.6 {65}[d]	
Monoterpenoids {43}[d]					40.9 ± 1.4 {40}[d]	29.5 ± 0.6 {42}[d]	
Sesquiterpenes {12}[d]					0.0 ± 0.0 [1][d]	1.8 ± 0.0 {12}[d]	
Sesquiterpenoids {10}[d]					3.7 ± 0.2 {9}[d]	1.6 ± 0.0 {9}[d]	
Other terpenoids {3}[d]					0.0 ± 0.0 [1][d]	0.1 ± 0.0 [1][d]	
Total phenylpropanoids and benzoic acid derivatives {8}[d]					16.3 ± 0.4 {8}[d]	5.0 ± 0.0 {7}[d]	
Total fatty acid derivatives {18}[d]					20.3 ± 0.7 {15}[d]	42.3 ± 1.1 {17}[d]	
Total Green leaf volatiles {6}[d]					18.2 ± 0.6 {6}[d]	40.2 ± 0.6 {6}[d]	
Others {55}[d]					9.4 ± 0.9 {47}[d]	10.7 ± 0.5 {53}[d]	
Total of identified compounds {149}[d]					90.5 ± 3.5 {119}[d]	90.9 ± 2.1 {142}[d]	

[a] Databases LRI shown in Table 1 for non-polar column are the closest value to experimental data; [b] LRI values on polar column obtained according to standards of n-alkanes, unless not available and specified otherwise; [c] tr: Relative proportion is marked as trace (tr) for values < 0.1%; [d] numbers in {} represent the number of identified compounds in that class; [e] LRI in FFNSC 3 database[54]; [f] LRI in Robert P. Adams library[55]; [g] LRI in NIST 14 database[56]; [h] FAEEs LRI are used instead of unavailable n-alkanes LRI); [i] identification with LRI on non-polar column; [j] identification with LRI on polar column.

Conclusions

Herein, we presented the first investigation on the volatile secondary metabolites from the leaves of *Betula glandulosa* MICHX., an abundant birch shrub in Nunavik that is an important actor in the ongoing shrubification phenomenon occurring in northern regions. No essential oil was retrieved from our steam distillation. However, the hydrosol obtained was extracted to study the volatile fraction and thoroughly characterized by GC-MS and GC/FID. Its volatile fraction contained various natural products and differed from the most common compositions for *Betula* essential oils or volatile emissions, even though the main compounds did show some similarities with previously published compositions for some birch species. The main compounds observed in *B. glandulosa* leaves' volatile samples were linalool, GLVs (mainly (*Z*)-3-hexen-1-ol), eugenol, and α-terpineol. The volatile composition of *B. glandulosa* could

be used in chemotaxonomy in the future, following more characterization of the volatile fraction of the different birch species. Further studies on this high-impact shrub from northern environments could lead to interesting information and help better predict future outcomes of the increasing presence of this shrub. The results helped us to understand specific birch defense mechanisms that may affect fauna and flora in the future as this species becomes more abundant in northern regions. Overall, the present investigation illustrates the importance of phytochemical investigations in northern regions, where specific biotic and abiotic factors can influence the production of secondary metabolites by organisms.

Experimental Section

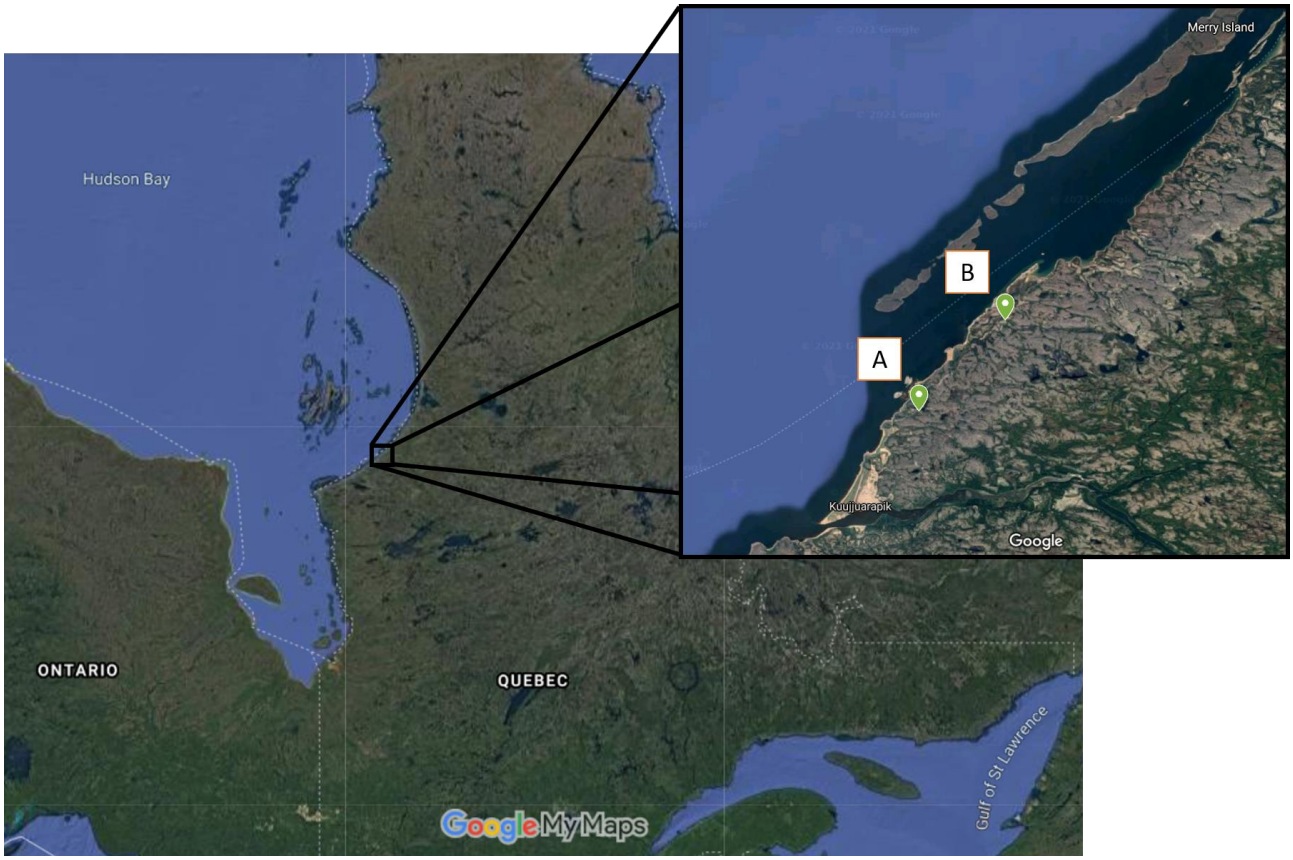

Figure 4. Location of harvest sites in the Whapmagoostui-Kuujjuarapik region, in Nunavik, Quebec, Canada.[57]

Plant material

Two samples of *Betula glandulosa* leaves were harvested on July 15 and July 17 of 2019 from two sites near the Nunavik communities of Whapmagoostui-Kuujjuarapik (N55°19'01,9" W77°43'00,3" and N55°21'28.9" W77°38'55.9"), as shown on Figure 4. Whapmagoostui-Kuujjuarapik is located on the Hudson Bay, in the forest subzone of the forest tundra ecotone.[58] On both sites, sampling was done randomly on shrubs found in an area of about 100 m diameter with a fairly uniform vegetation type. Plants were identified by Stéphane Boudreau, professor of the Département de biologie at Université Laval, and voucher specimens were deposited at the Louis-Marie Herbarium of Université Laval (# QFA0636315 and QFA063631). Only the leaves were harvested, placed in plastic bags, and stored at –20 °C upon arrival at the laboratory facility on the same day. Before distillation, the leaves were meticulously sorted to remove all other plant material (branches, buds, leaves of other species, etc.). Steam distillation was conducted within 6 days following collection.

Extraction

Betula glandulosa leaves were extracted as fresh plant material on July 20th, 2019, after thawing and sorting. 1726 g and 1474 g of fresh leaves were used to fill the distillation apparatus for Samples A and B respectively (dry mass was not determined since the extraction method does not allow an interesting yield calculation). The steam distillation apparatus was a portable alembic system developed by our lab and its description and photo is available in the supplementary information. *Betula glandulosa* leaves were steam distilled for 3 h with cohobation. A hydrosol was collected (225 mL) and stored at 4 °C until extraction. Liquid-liquid extraction of the organic phase followed 10 days later. The hydrosol was extracted thrice with purified ether (3 X 100 mL) (purified using VAC 103991 Solvent Purification System), dried with magnesium sulfate, and the solvent was evaporated on a rotatory evaporator, with a water bath at room temperature, to obtain an oily residue. The samples obtained were stored in glass vials at 4 °C and protected from light until analysis.

Identification of compounds

Volatile samples were diluted to about 10% w/w with purified CH_2Cl_2 (purified using VAC 103991 Solvent Purification System), and sample compounds were identified using a Thermo Scientific GC-MS (Trace GC Ultra with DSQ II detector) connected with a TriPlus autosampler (Thermo Scientific). For non-polar phase analyses, the column used was a DB-5MS 30 m X 0.25 mm X 0.25 µm while an HP-INNOWAX 30 m X 0.25 mm X 0.25 µm column was used for polar phase analyses. The carrier gas was helium, at a flow rate of 1.0 mL/min, in constant flow mode. The injector temperature was set to 200 °C, the injection volume was 1.0 µL, and the split ratio was set to 10. The temperature program was set as follows: 50 °C for 5 min, then increasing at 2 °C/min to 270 °C and held at this final temperature for 5 min. The mass range was 50–500 Da. The ionization energy was 70 eV. Thermo Scientific software: Excalibur was used for instrument control and acquisition, and Thermo Scientific's QualBrowser was used for processing. Compounds were identified by comparing their mass spectra and linear retention indices to GC-MS commercial spectral libraries (FFNSC 3 (Wiley)[54], Robert P. Adams' library[55] and NIST 14[56]). The alkane standard for linear retention index determination was a C_7–C_{30} saturated solution of alkanes (Millipore Sigma, 49451-U) for both columns. C_4-C_{24} even-numbered carbon FAEE and FAME standards (Millipore Sigma, 49454-U and 49453-U) were used for polar column analysis (LRIs according to FAME and FAEE are presented in the supplementary information). Linear retention indices were calculated using the equation from Van den Dool and Kratz[59].

The relative percentages of each compound in the total extracts were calculated with data obtained from GC-FID analyses and peak integration without correction factors. The GC/FID instrument was a Trace GC Ultra (Thermo Scientific) with a TriPlus autosampler (Thermo Scientific). The column was a DB-5MS, 30 m X 0.25 mm X 0.25 µm, the FID temperature was 300 °C (gas flows: air = 350 mL/min; H_2 = 35 mL/min; N_2 (makeup) = 30 mL/min), and the injection port temperature was 220 °C. The temperature program and other settings were used the same as for non-polar phase GC-MS analyses.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbdv.202100871R1.

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Author Contribution Statement

J.-C. S. performed the sampling, distillations, extractions, samples analyses, and data analyses, and wrote the manuscript. X. F. contributed to the design of the research plan and to the revision of the manuscript. S. B. contributed to the design of the research plan, sampling, and revision of the paper. N. V. conceived the project, discussed the results, and contributed to the sampling and to the revision of the manuscript.

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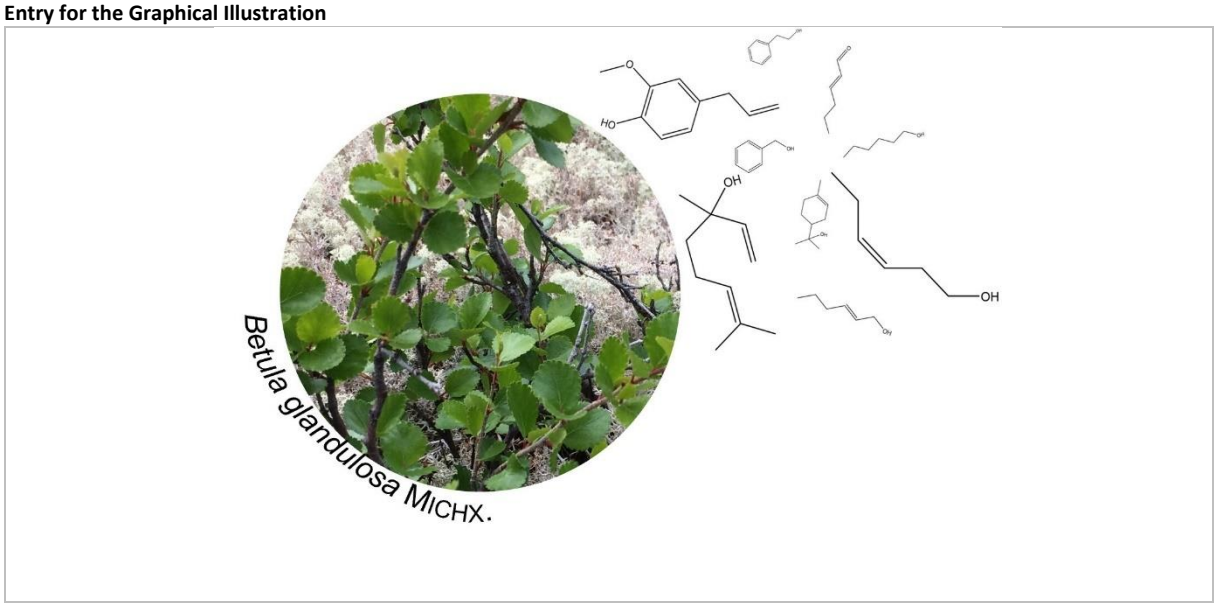

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Identification of Linalool, Green Leaf Volatiles, Eugenol and α-Terpineol as Main Compounds in the First Investigation of the Volatile Fraction of *Betula glandulosa*, a Prevalent Birch in Nunavik, by J.-C. Séguin et al., Université Laval, @nvoyerCHM.