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Chemical Composition and Antiplasmodial Activity of the Essential Oil of *Rhododendron subarcticum* Leaves from Nunavik, Québec, Canada

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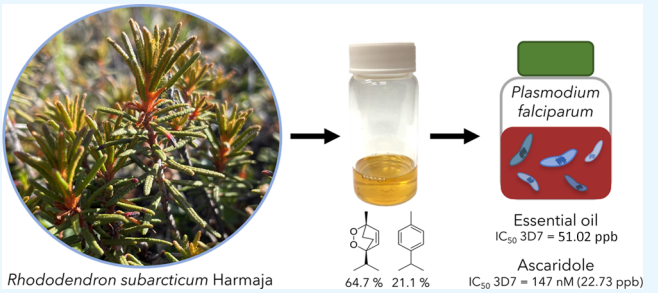
ABSTRACT: Dwarf Labrador tea, *Rhododendron subarcticum* Harmaja, is a popular medicinal plant in use by First Nations of Northern Canada, but its phytochemistry has remained largely unexplored. We have isolated and characterized the essential oil from a population of this species harvested near the treeline in Nunavik, Québec. Analyses by gas chromatography−mass spectrometry (GC−MS) and gas chromatography/flame-ionization detection (GC/FID) led to the identification of 53 compounds; the main secondary metabolites were ascaridole (64.7% of the total FID area) and *p*-cymene (21.1%). Such a composition resembles a chemotype observed for *R. tomentosum*, a close relative found mainly in Europe and Asia, but has never been attributed to *R. subarcticum*. Growth inhibition assays against different strains of *Plasmodium falciparum* (3D7, Dd2), the parasite responsible for the most severe form of malaria, were conducted with either the *R. subarcticum*'s essential oil or the isolated ascaridole. Our results show that the essential oil's biological activity can be attributed to ascaridole as its IC_{50} is more than twice that of ascaridole [ascaridole's IC_{50} values are 147.3 nM (3D7) and 104.9 nM (Dd2)].



INTRODUCTION

Plant species from the arctic and subarctic ecosystems have not been subjected to as many or as thorough phytochemical investigations as their counterparts from tropical environments. Dwarf Labrador tea (*Rhododendron subarcticum* Harmaja), an evergreen dwarf shrub, is a northern vascular plant whose phytochemistry remains largely unexplored. Formerly known as *Ledum palustre ssp. decumbens*, dwarf Labrador tea is one of the most commonly used medicinal plants by Inuit[1−3] and First Nations, including the Cree,[1,4,5] Dene,[4] and Chipewyan[4] nations in Canada.[1] This plant is known as *mamittuqutiit*,[3] *qijuktaaqpait*,[2] *tirluapik*, *tirlualuk*, *mamaittuqutik*, *tirluk*, *qisiqtuut*, *kjaussat*, or *mamaittuqutirlaq*[1] by North East Canada Inuit or *wiisichipukw* or *uschiischipakw* by Cree people.[5] It has been used to treat cold, flu, stomach problems, headaches, nasal congestion, chest pain, tuberculosis, and diabetes.[1,2,4] It is also considered to be good for overall health.[2] Traditional medicine used either the leaves or the whole plant, but some specific remedies used only the shoots and the roots.[1,2,4] Archeologists have also confirmed that Inuit consumed dwarf Labrador tea in Labrador during the 18th century.[6] Despite *R. subarcticum*'s importance in traditional medicine, its essential oil has not been evaluated for any biological activity. However, essential oils of closely related species (*L. palustre* and others) have demonstrated various biological activities of interest[7] like antifungal,[7,8] antimicrobial,[8,9] antioxidant,[7,9,10] antibacterial,[9] anti-inflammatory,[11] insect repellent,[12,13] antiproliferative,[14] and proapoptotic.[14]

Interestingly, several essential oils have demonstrated significant activities against parasites responsible for leishmaniasis,[15] amoebiasis,[16] Chaga's disease,[17] or malaria.[18] Malaria is an important health issue for young children in many tropical regions but especially in Africa.[19,20] Some plants have been reported to have important antiplasmodial activity, such as *Artemisia annua*, a plant that has been used for the treatment of malaria in China for centuries. One of its secondary metabolites, artemisinin, is an endoperoxide sesquiterpenoid now used as a drug, along with its derivatives.[21] However, since some *Plasmodium* strains are now resistant to artemisinin,[19] there is a constant need for new treatments because multidrug-resistant strains of *Plasmodium falciparum* and *P. vivax*, the

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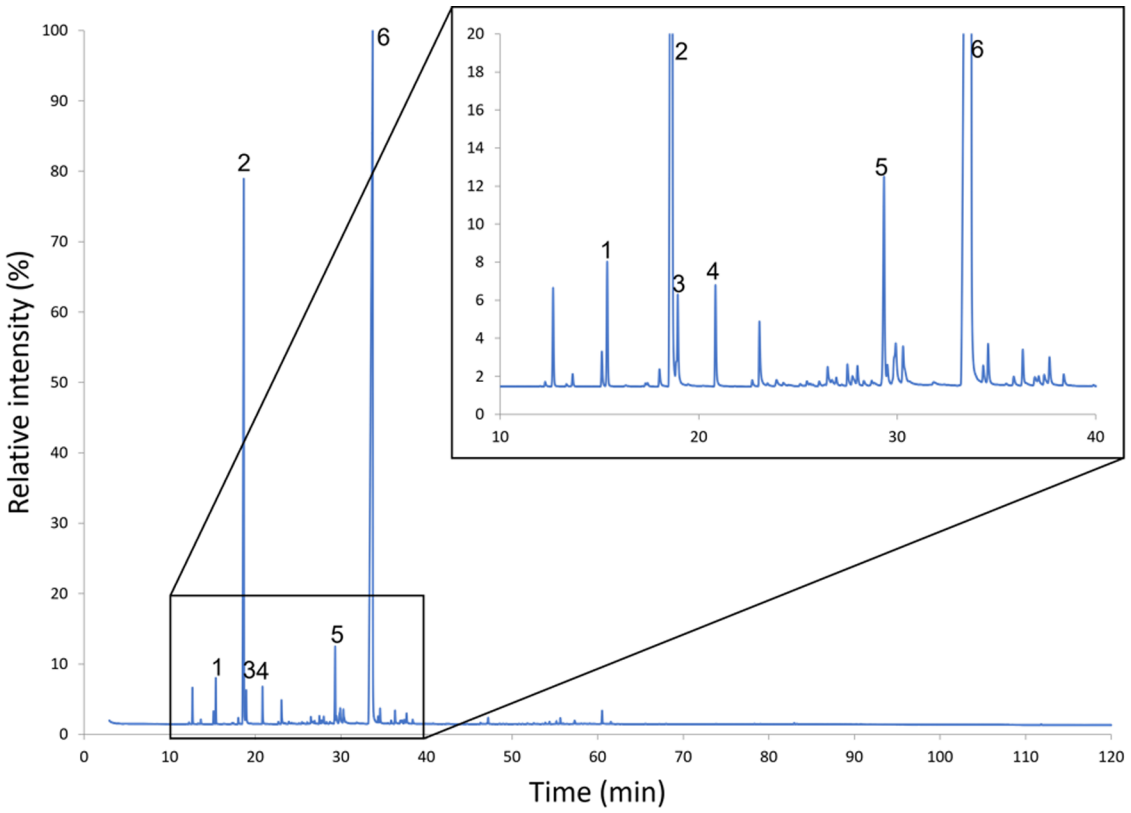

Figure 1. GC/FID chromatogram of *R. subarcticum* leaves' essential oil. 1, β-pinene; 2, *p*-cymene; 3, β-phellandrene; 4, γ-terpinene; 5, terpinen-4-ol; 6, ascaridole.

main parasites responsible for malaria, pose a great threat to human health.[22]

As the essential oil composition is greatly influenced by a variety of factors,[23] including environmental ones, investigating plant species growing under harsh climatic conditions at the forest−tundra ecotone in Nunavik (Québec) could provide new biologically active secondary metabolites or extracts. We decided to characterize the essential oil of *R. subarcticum* and test its activity against *P. falciparum* after learning of its importance for the First Nations as traditional medicine.

RESULTS AND DISCUSSION

R. subarcticum Harmaja is a dwarf evergreen shrub species that can grow up to 20 cm in height. Its leaves are very characteristic, with a dense hairy cover on the underneath surface.[24] As a taxonomically debated member of the *Rhododendron* genus of the *Ericaceae* family,[24] it has been considered closely related to *L. palustre*, either as a variety (*L. palustre* var. *decumbens* Aiton 1789) or a subspecies [*L. palustre* subsp. *decumbens* (Ait.) Hultén 1930]. It was also known as *L. decumbens* (Aiton) Lodd. ex Steud 1841 by some.[24−26]

In this study, we will consider *R. subarcticum* Harmaja as a taxon distinct, although very similar, to *R. tomentosum* Harmaja and as a synonym of the former *L. palustre* Linnaeus subsp. *decumbens* or *L. decumbens* (Aiton) Loddiges X Steudel, as per Harmaja (1990[26] and 1991[25]) and adopted by Gauthier.[24] To clarify the situation, Table S4 (Supporting Information) presents the studies on volatile extracts and essential oils from *L. palustre* or synonyms and close relatives.

Production and Characterization of the Essential Oil. The hydrodistillation of fresh *R. subarcticum* leaves harvested in the Boniface River region, in Nunavik, Québec, Canada, yielded 3.62 g (1.18%, calculated on the dry mass) of a yellow and very fragrant essential oil. The yield is within the range reported for *R. tomentosum* and synonyms.[27]

The essential oil was then analyzed by gas chromatography−mass spectrometry (GC−MS) and gas chromatography/flame-ionization detection (GC/FID) to identify and quantify the secondary metabolites. We identified 53 compounds, representing 98.8% of the essential oil GC area, in relative proportion. The GC/FID chromatogram of the extract (Figure 1) clearly shows that two compounds are particularly dominant, in major and minor proportions. The monoterpenoid endoperoxide ascaridole is the main secondary metabolite identified, comprising 64.7% of the essential oil. The second-most important compound in relative proportion was the monoterpene *p*-cymene (21.1%). Most of the essential oil was composed of monoterpenoids (27 compounds, 71.1%) and monoterpenes (18 compounds, 27.0%). All of the compounds that were identified, along with their linear retention indices (LRIs), relative proportions, and identification methods, are listed in Table 1, while the structures of the main compounds (>1%) are presented in Figure 2.

Literature is scarce about the volatile compounds of *R. subarcticum*, though other closely related species have been studied more. For example, the essential oil composition of *L. palustre ssp. decumbens* contained a high proportion of germacrone and myrcene. Interestingly, the essential oil of *L. palustre ssp. groenlandicum* from the same study had a very similar composition.[28] It was also reported that the volatile fraction from *L. palustre ssp. decumbens* from Alaska varied in quantity and composition across a latitudinal gradient and was influenced by resource availability, date of harvest, and phenological stage, as studied by static headspace. In this last

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Table 1. Composition of the Essential Oil from the Leaves of *Rhododendron subarcticum* Harmaja from the Boniface River Region in Nunavik

compounds	LRIs				relative proportion[a]	identification[b]
	(non-polar column)[c]		(polar column)[d]		[% ± SD]	
	exp.	lit.[e,f,g,h]	exp.	lit.[e,f,g,h]		
α-thujene*	922	924[e]	1028	1028[g]	0.1	MS, LRI[i,j]
α-pinene*	928	932[e]	1025	1028[g]	0.9 ± 0.1	MS, LRI[i,j]
camphene*	943	946[e]	1068	1071[g]	0.1	MS, LRI[i,j]
sabinene*	968	969[e]	1123	1124[g]	0.3	MS, LRI[i,j]
β-pinene*	972	974[e]	1112	1112[g]	1.2 ± 0.1	MS, LRI[i,j]
1-octen-3-ol	978	978[f]	1458	1450[g]	tr	MS, LRI[i,j]
myrcene*	987	988[e]	1170	1161[g]	tr	MS, LRI[i,j]
α-phellandrene*	1003	1002[e]	1165	1167[g]	tr	MS, LRI[i,j]
δ-3-carene*	1004	1009[f]	1148	1147[g]	tr	MS, LRI[i,j]
α-terpinene*	1013	1014[e]	1181	1180[g]	0.2	MS, LRI[i,j]
p-cymene*	1025	1025[f]	1273	1272[g]	21.1 ± 1.0	MS, LRI[i,j]
limonene*	1026	1030[f]	1202	1200[g]	0.2	MS, LRI[i,j]
β-phellandrene*	1027	1025[f]	1210	1211[g]	1.0 ± 0.1	MS, LRI[i,j]
(*Z*)-β-ocimene*	1034	1035[f]	1238	1235[g]	tr	MS, LRI[i,j]
(*E*)-β-ocimene*	1044	1044[e]	1254	1250[g]	tr	MS, LRI[i,j]
γ-terpinene*	1054	1054[e]	1245	1246[g]	1.0	MS, LRI[i,j]
terpinolene*	1080	1086[f]	1283	1283[g]	0.1	MS, LRI[i,j]
p-cymenene*	1085	1089[e]	1437	1444[g]	0.7	MS, LRI[i,j]
linalool*	1099	1101[f]	1557	1547[g]	0.1	MS, LRI[i,j]
n-nonanal*	1103	1100[e]	1396	1391[g]	tr	MS, LRI[i,j]
1,3,8-*p*-menthatriene*	1107	1108[e]	1422	1418[h]	tr	MS, LRI[i]
dehydro sabina ketone*	1112	1117[e]	1638	1643[g]	tr	MS, LRI[i,j]
cis-*p*-menth-2-en-1-ol*	1118	1118[e]	1626	1638[g]	tr	MS, LRI[i,j]
trans-pinocarveol*	1133	1135[e]	1651	1664[g]	0.1	MS, LRI[i,j]
trans-*p*-menth-2-en-1-ol*	1137	1138[e]	1562	1571[g]	0.2	MS, LRI[i,j]
camphor*	1138	1141[e]	1504	1520[g]	0.1	MS, LRI[i,j]
pinocarvone*	1154	1160[e]	1557	1586[g]	0.1	MS, LRI[i]
borneol*	1164	1165[e]	1698	1702[g]	0.1	MS, LRI[i,j]
terpinen-4-ol*	1176	1174[e]	1600	1602[g]	2.5	MS, LRI[i,j]
thuj-3-en-10-al*	1177	1181[e]	1619	1642[g]	0.3	MS, LRI[i]
cryptone	1179	1183[e]	1655	1687[g]	tr	MS, LRI[i]
myrtenal*	1187	1184[f]	1616	1647[g]	0.4 ± 0.4	MS, LRI[i]
α-terpineol*	1188	1186[e]	1698	1697[g]	0.4 ± 0.4	MS, LRI[i,j]
p-cymen-8-ol*	1190	1189[f]	1853	1852[g]	0.4	MS, LRI[i,j]
estragol*	1192	1195[e]	1668	1670[g]	0.2	MS, LRI[i,j]
myrtenol*	1194	1194[e]	1790	1797[g]	tr	MS, LRI[i,j]
ascaridole*	1245	1240[f]	1712	1889[g]	64.7 ± 0.7	MS, LRI[i]
cis-piperitone epoxide (epoxide vs IPP)*	1250	1250[e]	1720	1707[g]	0.3 ± 0.1	MS, LRI[i,j]
carvenone oxide	1254	1261[g]	1707		0.5 ± 0.1	MS, LRI[i]
phellandral*	1270	1273[e]	1707	1744[g]	0.1	MS, LRI[i]
bornyl acetate*	1278	1284[e]	1573	1581[g]	0.4	MS, LRI[i,j]
p-cymen-7-ol*	1287	1289[e]	2100	2113[g]	tr	MS, LRI[i,j]
thymol*	1290	1289[e]	2182	2189[g]	0.1	MS, LRI[i,j]
carvacrol*	1297	1298[e]	2222	2236[g]	0.2	MS, LRI[i,j]
isoascaridole*	1299	1306[f]	1851	1853[g]	0.3	MS, LRI[i,j]
p-mentha-1,4-dien-7-ol*	1322	1325[e]	2054	2073[g]	tr	MS, LRI[i,j]
citronellyl acetate*	1347	1350[f]			tr	MS, LRI[i]
geranyl acetate*	1375	1379[e]			tr	MS, LRI[i]
aromadendrene*	1447	1440[g]	1629	1618[g]	tr	MS, LRI[i,j]
(*E*)-β-farnesene*	1448	1452[f]			tr	MS, LRI[i]
caryophyllene oxide*	1566	1582[e]	1957	1989[g]	0.1	MS, LRI[i]
14-hydroxy-(*Z*)-caryophyllene*	1670	1664[f]			tr	MS, LRI[i]
germacrone*	1676	1693[e]	2202	2217[g]	0.4	MS, LRI[i,j]
terpenoids (50)[k]					98.6 ± 3.1	
monoterpenes (18)					27.0 ± 1.3	
monoterpenoids (27)					71.1 ± 1.8	
sesquiterpenes (2)					tr	
sesquiterpenoids (3)					0.5	

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Table 1. continued

compounds	LRIs	relative proportion[a]	identification[b]
others (3)		0.2	
total proportion of identified compounds (53)		98.8 ± 3.2	

[a]Relative proportion is marked as trace (tr) for values <0.1%. [b]Identification with the LRI is reported with specific column polarity. [c]Database LRIs shown in this table for the non-polar column are the closest value to experimental data. [d]LRI values on the polar column are obtained according to the standards of *n*-alkanes. [e]Data from Adams, 2017.[39] [f]Data from FFNSC 3.[40] [g]Data from NIST 14.[41] [h]Data from ESO 2000.[42] [i]LRI on the non-polar column. [j]LRI on the polar column. [k]Numbers in parentheses show the number of identified compounds in that class. Compounds reported in the literature in essential oils from *R. subarcticum*, *R. tomentosum*, or synonyms are marked with an asterisk.[7,9,11,12,27−38,43−52]

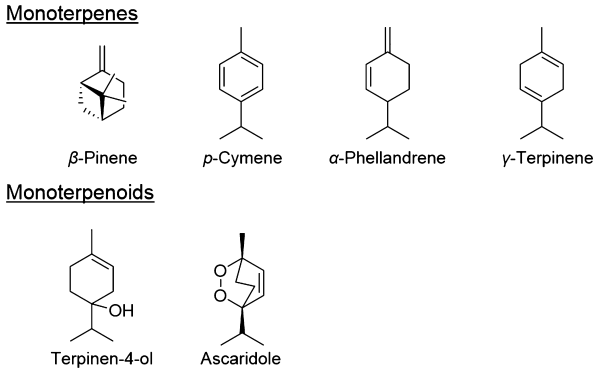

Figure 2. Main secondary metabolites (>1%) identified in the essential oil from *R. subarcticum* leaves from Nunavik.

study, the composition of *L. palustre ssp. decumbens* also included some monoterpenes and monoterpenoids, including high proportions of cymene.[29]

On the other hand, *R. tomentosum* has been much studied in Europe and Asia. A review of the available scientific literature on the essential oils of the former *L. palustre* L. reveals no less than 10 different chemotypes from various countries, excluding data from *R. subarcticum* or synonyms.[14] The most important compounds reported in essential oils of *L. palustre* are sabinene, *β*-myrcene, *p*-cymene, limonene, *α*-thujenal, *γ*-terpineol, bornyl acetate, ascaridole, palustrol, and ledol.[14] Ledol and palustrol were considered the typical constituents of *L. palustre* essential oil,[14,28,30,31] but other chemotypes are often observed, and these two typical sesquiterpenoids are sometimes found only in trace amounts or not even observed.[14,30] The review shows that there is a substantial variability in the essential oil composition of this species, even within the same country;[27] this variability could be due to differences in phenology[7,32] or age at the time of harvest.[33] The importance of the harvest time was also identified as an important driver of the variability of the *R. tomentosum* essential oil composition in Poland[34] and of polyphenols in *R. subarcticum*.[1] Other factors that affect the essential oil composition obtained from *R. tomentosum* include the part of the plant distilled,[35,36] geography,[34,37] drying,[34] and the isolation method.[34]

Our results from the *R. subarcticum* sampled in Nunavik differed substantially from the results reported for *L. palustre ssp. decumbens*. In fact, ascaridole, the dominant compound found in *R. subarcticum* essential oil, has never been reported in *L. palustre ssp. decumbens*,[28,29] although we did find a low quantity of germacrone and myrcene in our essential oil extract. Another compound, *p*-cymene, is quite abundant, although not the most dominant. However, a chemotype dominated by ascaridole and *p*-cymene has been identified for *R. tomentosum* essential oil in Lithuania,[30] Poland,[31] Russia,[14] Finland,[14] and China[38] and is one of the 10 known chemotypes for *R. tomentosum*.[14]

Ascaridole is a well-known monoterpenoid that is generally the main secondary metabolite in the essential oils of *Dysphanya ambrosioides*[53] and *Peumus boldus*.[54] Interestingly, for those two plants and for *R. tomentosum*, when ascaridole is abundant, it is found along with *p*-cymene, which is known as an ascaridole/*p*-cymene chemotype.[14,54] Ascaridole is also sometimes abundant in the essential oils from *Achillea filipendulina*,[55] *Achillea biebersteinii*,[56,57] *Croton regelianus*,[58] and *Artemisia persica*.[59] Its quantification by GC is known to be difficult because of its propensity to rearrange when heated above 150 °C to the form of isoascaridole.[53,60] Ascaridole rearrangement can occur during the injection in the GC system, caused by high temperatures at the inlet.[61] Some authors typically combine the relative proportions of both ascaridole and isoascaridole to report it as the total ascaridole content in the essential oil.[31] Some authors reported that a combination of GC and ^{13}C NMR allowed for a good quantification of the endoperoxide.[53]

We tested different inlet temperatures for the GC injections and learned that by lowering the inlet temperature to 170 °C, the degradation of ascaridole was quite low. We deduced this because only a small amount of isoascaridole was observed, compared to the amounts observed with higher temperatures (see Supporting Information, Figure S2 and Table S3).

Since it still allowed the observation of the other compounds, as with higher temperature injections, lowering the inlet temperature seems to be a good and simple way to limit ascaridole rearrangement to isoascaridole. It is possible to determine the relative proportion of ascaridole under those conditions, and very little isoascaridole is observed (0.3%), but it is not possible to know if there is isoascaridole in the essential oil or if it totally arises from ascaridole rearrangement when heated.

Among other major compounds in previously reported essential oils from *R. tomentosum*,[14] both marker compounds, ledol and palustrol, were not observed in the essential oil from *R. subarcticum* sampled in Nunavik. Other reported main compounds such as *α*-thujenal and *γ*-terpineol were not observed either; myrcene was present in trace amounts; and *α*-terpinene (0.2%), limonene (0.2%), sabinene (0.3%), and bornyl acetate (0.4%) were only present as minor compounds. Only one occurrence of the ascaridole/*p*-cymene chemotype was reported with no ledol or palustrol in Northeast China,[38] while in Poland, this chemotype was found with low levels of ledol but no palustrol.[31] Other reports of this chemotype all identified ledol and palustrol among the observed secondary metabolites.[14,30] In some cases, the presence of ascaridole has been linked to the phenological stage, with ledol and palustrol being more abundant in early and late growth season and ascaridole increasing in proportion in July and August.[7,32]

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The essential oil was fractionated on a silica gel column with a hexanes/methylene chloride eluent system, and fractions 25−31 contained over 90% ascaridole [sum of ascaridole and isoascaridole in GC/FID is 93.0%, and no isoascaridole signal is observed in nuclear magnetic resonance (NMR)]. This fraction was characterized to properly identify ascaridole as the main compound of the essential oil and of this fraction. Ascaridole characterization and structure confirmation were done by GC−MS, polarimetry, and NMR (^{1}H, ^{13}C, COSY, HSQC, HMBC, and DEPT) (see data below). The NMR spectra are presented in the Supporting Information. The experimental polar LRIs we observed for ascaridole are quite different from the reference in the NIST 14 library; no polar LRI was to be found in the Robert P. Adams, FFNSC 3, or ESO 2000 libraries. A polar LRI similar to what we observed was reported by Cavalli et al., 2004 (1715),[53] but other authors[31,36,62,63] reported LRI values closer to what is documented in NIST 14 or in resources considered by NIST 14. The values available for polar column LRIs are limited compared to the available non-polar column LRI values. With our identification by NMR and other GC and GC−MS analyses, we are confident that the compound is ascaridole and that the reported polar column LRI is accurate. The fractionation allowed the observation of isoascaridole in low amounts in the fractions that did not contain any ascaridole (fractions 41−44), confirming that the essential oil does contain low amounts of this compound.

Antiparasitic Activity Assays. Like *R. subarcticum* and *R. tomentosum*, *D. ambrosioides* and *P. boldus* are both important medicinal plants for Indigenous people who live where they grow.[54,64−66] Part of their biological activity is associated with the ascaridole content.[54,67,68] In particular, *D. ambrosioides* is an important traditional medicine for the treatment of various diseases and afflictions linked to parasites. Its essential oil, rich in ascaridole, has shown important activity against protozoan parasites including the *Plasmodium* species.[15,16,18,67,69−71] Research on ascaridole alone has shown that it has great potential against parasites responsible for malaria, leishmaniasis, Chaga's disease, and amoebiasis.[16,17,67,72] Synergistic effects have also been observed, showing that mixtures of compounds, particularly ascaridole and carvacrol, can have a more potent effect than pure compounds against *Leishmania*.[71] Even though ascaridole has been identified as a main compound of one chemotype of *R. tomentosum*, this shrub has never been tested against such parasites.

In an in vivo investigation of the antimalarial activity of ascaridole and other endoperoxides, it was reported that ascaridole was not active at the maximum tolerated dose.[73] In vitro assays later showed that the natural endoperoxide was active against *P. falciparum* and inhibited growth of the parasites at a 0.05 μM concentration, while it eliminated the parasites at a 0.1 μM concentration.[74] In another report, ascaridole's IC_{50} was evaluated at 650 nM against *P. falciparum* (NF54), but the authors reported that the activity might be underestimated due to the volatility of the metabolite.[75] The activity of the essential oil of *D. ambrosioides* was measured against protozoan parasites and was quite high against *P. falciparum*, even though its main metabolites (ascaridole, carvacrol, and caryophyllene oxide) did not show activity in their assays.[67] The same research team also reported that the inhibition activity of the essential oil against *Leishmania amazonensis* was higher than that of pure compounds and that synergistic effects might be involved between ascaridole and carvacrol.[15,71] The toxicity of the essential oil and some pure compounds mixed in different ratios was lower than that of pure compounds as well, while keeping an interesting activity against the parasite.[67,71]

In our studies, the isolated ascaridole and the essential oil of *R. subarcticum* were tested against two strains of *P. falciparum*, the parasite responsible for the most severe form of malaria. The strains used were the reference strain 3D7 and the multidrug-resistant strain Dd2.[76,77] The ascaridole fraction was moderately active against the parasites and demonstrated an IC_{50} of 147.3 ± 7.3 nM against 3D7 (n = 4) and 104.9 ± 11.2 nM against Dd2 (n = 4), as shown in Table 2. The essential oil was active against the two *P. falciparum* strains as well, but the comparison of the growth inhibition by the ascaridole fraction with that of *R. subarcticum* essential oil suggests that the antiplasmodial activity of the essential oil almost entirely comes from its main compound as the IC_{50} values in ppb (v/v) were 22.73 ± 1.13 (3D7, n = 4) and 16.14 ± 1.73 (Dd2, n = 4) for the ascaridole fraction and 51.02 ± 3.24 (3D7, n = 4) and 42.13 ± 3.15 (Dd2, n = 4) for the essential oil. These results show that ascaridole has an IC_{50} value 55% lower against 3D7 and 62% lower against Dd2 than the essential oil containing 64.7% of the endoperoxide monoterpenoid.

Table 2. IC_{50} Measured against 3D7 and Dd2 *P. falciparum* Strains for the Ascaridole-Rich Fraction and *R. subarcticum* Essential Oil

	ascaridole fraction IC_{50} (nM)		ascaridole fraction IC_{50} (ppb; v/v)		essential oil IC_{50} (ppb; v/v)	
strain	mean	± SEM	mean	± SEM	mean	± SEM
3D7	147.3	7.3	22.73	1.13	51.02	3.24
Dd2	104.9	11.2	16.14	1.73	42.13	3.15

We identified low amounts of carvacrol (0.2%) in the essential oil of *R. subarcticum*, which is probably insufficient for a synergistic effect. With the activity reported for the essential oil, no compound in the extract seems to have that kind of effect either.

In summary, we described the first characterization of the essential oil from *R. subarcticum* leaves from specimens from a subarctic ecosystem, Nunavik, Québec, Canada. This rare phytochemical investigation of this taxon demonstrated that *R. subarcticum* can be compared with its morphologically close relative, *R. tomentosum*, with the identified ascaridole/*p*-cymene chemotype being one of the reported chemotypes for European and Asian samples of *R. tomentosum*. Overall, our results show the potential of *R. subarcticum* natural products in the treatment of parasitic diseases, especially certain drug-resistant strains of *P. falciparum*. It also showed the importance of phytochemical investigations of sources from largely unexplored northern ecosystems.

EXPERIMENTAL SECTION

Plant Material. Leaves of *R. subarcticum* were harvested in August 2019 from sites near the Boniface River research station of the Centre d'études nordiques (Laval University), in Nunavik (N 57°45′03″ W 76°10′24″; https://www.cen.ulaval.ca/en/station.php?id=327&nm=boniface). The Boniface River research station is located ca. 10 km south of the treeline in the forest−tundra ecotone.[78] Sampling was done on randomly selected individuals previously identified by S. Boudreau (Département de biologie, Université Laval). A voucher

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specimen was deposited at the Louis-Marie herbarium at Université Laval (# QFA0642460). Aboveground biomass, mainly leaves, was harvested, placed in plastic bags, and frozen within 6 h from harvest. Before distillation, leaves were meticulously sorted to remove all other plant material (branches, buds, other species leaves, etc.).

Extraction. Once thawed, the essential oil was extracted through the hydrodistillation of fresh *R. subarcticum* leaves. The dry mass of leaves used for distillation was evaluated to be 307 g. The distillation yield was calculated using the measured dry mass. Hydrodistillation was done using a Clevenger-type apparatus (see the picture and description in the Supporting Information, Figure S1). Leaves were distilled for 3 h with cohobation. The essential oil was then recovered with a Pasteur pipette, dried over magnesium sulfate, and stored in the dark at 4 °C in a glass vial until further analysis.

Identification of Compounds. The essential oil extract was diluted to ca. 10% w/w with purified CH_2Cl_2 (purified using a VAC 103991 solvent purification system) to identify its compound with a Thermo Scientific GC−MS (Trace GC Ultra with a DSQ II detector) connected to a TriPlus autosampler (Thermo Scientific). For the non-polar and polar phase analyses, we used a DB-5MS 30 m × 0.25 mm × 0.25 μm column and an HP-INNOWAX 30 m × 0.25 mm × 0.25 μm column, respectively. The carrier gas was helium, at a flow 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, increasing to 270 °C at a rate of 2 °C/min, and held at this final temperature for 5 min. The mass range was 50−500 Da. The ionization energy was 70 eV.

Data acquisition, instrument control, and processing were achieved with Thermo Scientific software (Excalibur for instrument control and acquisition and QualBrowser for processing). Compounds were identified by comparing mass spectra and LRIs to GC−MS commercial spectral libraries (FFNSC 3 (Wiley),[40] Robert P. Adams' library,[39] NIST 14,[41] and ESO 2000[42]). The alkane standard for LRI determination was a C7−C30 saturated alkane solution (Millipore Sigma, 49451-U) for both columns. C4−C24 even carbon numbered fatty acid ethyl esters (FAEEs) and fatty acid methyl esters (FAMEs) standards (Millipore Sigma, 49454-U and 49453-U) were also used for polar column analysis. LRIs were calculated using the equation by Van den Dool and Kratz.[79]

The relative percentages of compounds in 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 DB-5MS, 30 m × 0.25 mm × 0.25 μm. The FID temperature was 300 °C [gas flows: air = 350 mL/min; H_2 = 35 mL/min; and N_2 (makeup) = 30 mL/min], and the injection port temperature was 170 °C. The temperature program and other settings were the same as used for non-polar phase GC−MS analysis.

The analysis of fractions recovered from flash chromatography was performed as described above for the essential oil sample with the following changes: a Trace Ultra GC−MS was used with an ITQ 900 MS detector, with a gradient from 50 (5 min hold time) to 250 °C (5 min hold time) at a 10 °C/min rate. Fractions were injected directly from the tube contents after flash chromatography.

NMR analyses were used to confirm the identification of ascaridole, the main compound, using a 500 MHz DD2 NMR (Agilent Technologies) with $CDCl_3$ as the solvent (Millipore Sigma). Chemical shifts (δ) are reported in ppm as a function of the residual signal of $CDCl_3$ (δH = 7.26 ppm). Coupling constants are reported in Hz. Multiplicity patterns are described as s (singlet), d (doublet), sept (septuplet), and m (multiplet).

Optical rotation was measured using a Jasco DIP-360 digital polarimeter with a sodium lamp, at a wavelength of 589 nm, at room temperature (around 25 °C), with a concentration of 0.03 g/mL in chloroform, using a 10 cm long cell.

Ascaridole. Pale yellow oil; $[\alpha]_D^{25} = -0.03°$ (0.03 g/mL, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.00 (6H, d, J = 7 Hz), 1.37 (3H, s), 1.45−1.58 (2H, m), 1.92 (1H, sept, J = 7; 6.95; 6.9 Hz), 1.97−2.11 (2H, m), 6.41 (1H, d, J = 8.5 Hz), 6.49 (1H, d, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 17.1 (CH_3), 17.2 (CH_3), 21.4 (CH_3), 25.6 (CH_2), 29.5 (CH_2), 32.1 (CH), 74.3 (C), 79.8 (C), 133.0 (CH), 136.4 (CH); EIMS m/z = 121 (100), 93 (72), 136 (59), 55 (26), 79 (24), 97 (22), 91 (21), 107 (17), 92 (15), 77 (15), $[M]^+$ = 168.05. Optical rotation and spectral data were in accordance with previous reports.[17,39,53,80]

Flash Chromatography Fractionation. Fractionation of *R. subarcticum* essential oil was done by flash chromatography on a silica gel column (Silicycle, SiliaFlash P60, 40−63 μm) using a hexanes/methylene chloride (both purified using the VAC 103991 solvent purification system) gradient (50 mL each: 90/10, 70/30, 50/50, 20/80, and 0/100). The essential oil (500 mg) was fractionated on a 30 g silica gel column, and fractions were collected in 5 mL volumes. Fractions 25−31 contained over 90% ascaridole, as determined by GC/FID.

Parasite Culture. *P. falciparum* 3D7 and Dd2 strains were obtained through BEI Resources, NIAID, NIH, contributed by Daniel J. Carucci and David Walliker (# MRA-102 and # MRA-150, respectively). Asexual-stage *P. falciparum* parasites were cultured in vitro under standard conditions in RPMI-1640 (Gibco)-HEPES-buffered medium supplemented with 0.5% (w/v) Albumax (Invitrogen) at 4% hematocrit (human red blood cells of O+ group). Parasites were kept at 37 °C in a gas mixture composed of 5% oxygen, 5% carbon dioxide, and 90% nitrogen. Parasite synchronization was done using a D-sorbitol treatment, as previously described.[81]

In Vitro 72 h Susceptibility Assays. Parasite susceptibility was measured as previously described, with minor modifications.[82] To avoid the volatile toxic effects of the essential oil extracts, the assays were performed in T25 unventilated flasks that were gassed individually for 10 s. Briefly, sorbitol-synchronized 4 to 12 h post-invasion ring-stage parasites were exposed to a 13-point serial dilution of each extract at 1% hematocrit and 0.5% starting parasitaemia in a 5 mL final volume. Cells were incubated for 72 h before 100 μL of resuspended culture was transferred in triplicate into 96-well plates. Cells were then disrupted, and the released DNA was stained by adding 25 μL of 5× lysis buffer containing 0.16% saponin, 20 mM Tris−HCl pH 7.5, 5 mM EDTA, 1.6% Triton X-100, and 5× SYBR Gold (Invitrogen). The plates were sealed and allowed to rest at room temperature for 24 h. Relative fluorescence units (RFUs) were measured with a VICTOR plate reader (PerkinElmer) at an excitation of 494 nm and an emission of 530 nm. RFU values were compared to those of untreated parasites, and data were plotted using GraphPad PRISM software, and IC_{50} values were determined using the curve-fitting algorithm *log(inhibitor) versus response−variable slope*.

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■ ASSOCIATED CONTENT

Supporting Information

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Distillation apparatus; GC and MS information on unidentified compounds; retention indices on the polar column; GC/FID chromatograms and relative proportions observed by analysis with GC/FID at different inlet temperatures; taxonomy in previously reported volatile extracts and essential oils; distribution of IC50 results for ascaridole; ascaridole NMR spectra; and inhibition activity graphs of ascaridole (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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