American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
AJEONP

ISSN: 2321 9114
AJEONP 2014; 2 (2): 17-24

Received: 10-08-2014
Accepted: 19-01-2015

Chelsey D. Stewart
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

Chelsea D. Jones
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

Correspondence:
William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.
wsetzer@chemistry.uah.edu

Essential oil compositions of *Juniperus virginiana* and *Pinus virginiana*, two important trees in Cherokee traditional medicine

Chelsey D. Stewart, Chelsea D. Jones and William N. Setzer

Abstract
The essential oils from the leaves and barks of *Juniperus virginiana* and *Pinus virginiana*, two coniferous trees important in Native American traditional medicine, were obtained by hydrodistillation. The essential oil from the "berries" of *J. virginiana* was also obtained. The essential oils were analyzed by gas chromatography – mass spectrometry. *J. virginiana* bark oils were dominated by α-pinene, while the leaf oils were rich in safrole, methyl eugenol, and elemol. *J. virginiana* berry essential oil was dominated by limonene and elemol. Both the bark and the leaf essential oils from *P. virginiana* had high concentrations of α-pinene, β-pinene, and β-phellandrene. The essential oils were screened for antibacterial and antifungal activity, but showed only marginal activity. The high concentrations of limonene in the berries, α-pinene in the bark, and safrole and methyl eugenol in the leaves of *J. virginiana,* and the large quantities of α- and β-pinenes and β-phellandrene in *P. virginiana* likely account for the traditional uses of these plants.

Keywords: Eastern red cedar, Virginia pine, chemical composition, essential oil.

1. Introduction
Like native cultures throughout the world, Native Americans relied on plants as their primary source of medicines. Thus, for example, the *Cherokee* people of the southeastern United States used the roots of *Aristolochia serpentaria* (Virginia snakeroot) to treat snakebite as well as to prepare a tonic for colds and fevers, a gargle for sore throat, and as a diuretic and diaphoretic; *Adiantum pedatum* (maidenhair fern) was used to make a poultice for rheumatism and chills; and the root of *Panax quinquefolius* (ginseng) was used to make a tonic [1, 2, 3].

Juniperus virginiana L. (eastern red cedar), Cupressaceae, is a medium-sized, dioecous, aromatic conifer ranging in the eastern United States from Michigan, south to Florida, and west to Oklahoma and Kansas [4, 5]. The tree was used by the *Cherokee* as a diaphoretic, as a tea for colds and measles, as an ointment for itch and cutaneous disease, and the berries were used against worms [2]. The Alabama, Creek, and Seminole Native Americans used *J. virginiana* externally to treat rheumatic pains, while an infusion of the leaves was used by the Creeks and Seminoles to treat colds and fever [6]. European-Americans in the Ozark-Ouachita Highlands (northwestern Arkansas and southwestern Missouri) used the berries of *J. virginiana* to treat edema, bronchitis, and heartburn [7]. *Juniperus virginiana* wood essential oil (cedarwood oil) has become a commercially important product [8-10] and has been extensively studied [11, 12].

Pinus virginiana Miller (Virginia pine), Pinaceae, is a small to medium-sized conifer that ranges from Pennsylvania, south through the Appalachian Mountains to western Tennessee and Alabama [4, 5]. *P. virginiana* was used to make a wash for skin ulcers and sores, the sap was used on stubborn sores that had difficulty healing [3], the inner bark was used for expelling intestinal worms and parasites, a syrup made from the bark was used as an expectorant for treating congestion and coughs, rheumatism, and venereal disease [2, 3]. *P. virginiana* oil was used for colds and bathing painful joints, while a tea from the needles was used for fever and colds [2].

In this work, we present the chemical compositions of *Juniperus virginiana* leaf and bark essential oils from both male and female trees, As well and the "berry" essential oil. We also present the essential oil compositions of the leaf and bark oils from *Pinus virginiana*.

2. Materials and Methods

2.1 Plant Material

J. virginiana samples were collected from several mature trees growing on the campus of the University of Alabama in Huntsville on May 06, 2014, and were identified by W.N. Setzer. Samples of *P. virginiana* were collected from several mature trees growing in Franklin County, Alabama on January 20, 2014, and were identified by Jeff K. Stewart, Forestry Consultant. Plant materials were each hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with $CHCl_3$ to obtain the essential oils (Table 1).

2.2 Gas Chromatography – Mass Spectrometry

GC-MS analyses of the essential oils were carried out using an Agilent 6890 GC with Agilent 5973 mass selective detector as previously described [13]. Identification of the oil components was achieved based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [14] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Antimicrobial Screening

The *J. virginiana* and *P. virginiana* essential oils were screened for antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* using the microbroth dilution technique as previously described [15].

Table 1: *Juniperus virginiana* and *Pinus virginiana* essential oil yields.

Plant Material	Mass of Plant Material (g)	Essential Oil Yield (mg)	Description of Oil
J. virginiana			
bark, male	110.18	1812.2	Pale yellow
bark, female	165.76	2947.1	Pale yellow
leaves, male	269.49	3132.8	Clear, colorless
leaves, female	445.01	3442.6	Clear, colorless
"berries"	232.91	890.4	Clear, colorless
P. virginiana			
bark	203.41	2745.1	Pale yellow
leaves	255.10	294.2	Pale yellow

3. Results and Discussion

The essential oils from the inner barks were obtained in relatively high yields. *J. virginiana* gave 1.64% and 1.78% bark oils for the male and female trees, respectively, while *P. virginiana* bark oil was obtained in 1.35%. The leaf oils for *J. virginiana* were obtained in 1.16% and 0.774%, respectively, for male and female trees, and the leaf oil yield of *P. virginiana* was 0.115%. *J. virginiana* berries gave an essential oil in 0.382% yield.

The essential oil compositions of *J. virginiana* are compiled in Table 2. A total of 65 compounds accounting for 98.0% of the composition and 73 compounds accounting for 97.8% of the composition were identified in the bark essential oils of male and female *J. virginiana*, respectively. Sixty-six compounds (96.5%) were identified in the leaf oil of male *J. virginiana*, while 68 compounds (99.8%) were identified in the female leaf oil. The essential oil from the berries of *J. virginiana* was composed of 33 compounds (100%). The differences in essential oil compositions between male and female trees were marginal.

J. virginiana bark oils were dominated by α-pinene (77.4% and 77.5% for male and female, respectively). The leaf oils, on the other hand, were composed largely of safrole (18.8% and 22.3%, respectively), methyl eugenol (13.8% and 11.9%, respectively), and elemol (10.6% and 13.6%, respectively). In contrast, the essential oil from *J. virginiana* berries was rich in limonene (63.1%) and elemol (18.4%). This leaf oil composition, as revealed in this present study, is qualitatively similar to previous studies [16-19]. The essential oil from the "berries" of *J. virginiana* in this study was similar in composition to that reported from a sample grown in Romania [19] with the exception that the Romanian sample had a large concentration of β-phellandrene (12.4%), which was not observed in our sample from Alabama. The Alabama sample showed a very large concentration of limonene (63.1%), however.

Table 2: Chemical compositions of *Juniperus virginiana* essential oils (average of three measurements ± standard deviations).

RI[a]		Bark		Leaf		Berries
	Compound	Male	Female	Male	Female	
926	Tricyclene	---	---	tr	tr	---
932	α-Thujene	---	---	tr	0.3±0.0	tr
940	α-Pinene	77.4±0.4	77.5±0.9	6.5±0.4	2.3±0.1	0.2±0.0
954	Camphene	0.4±0.0	0.3±0.0	0.6±0.2	0.1±0.0	---
958	α-Fenchene	0.1±0.0	0.1±0.0	---	---	---
961	Thuja-2,4(10)-diene	0.2±0.0	0.1±0.0	---	---	---
977	β-Pinene	1.1±0.0	1.3±0.1	---	---	0.2±0.0

976	Sabinene	0.8±0.0	0.9±0.1	2.8±1.8	8.7±0.2	---
984	1-Octen-3-ol	---	---	0.2±0.1	0.3±0.1	---
992	Myrcene	1.5±0.0	3.0±0.1	0.4±0.0	0.5±0.2	1.5±0.0
1003	α-Phellandrene	---	---	tr	tr	---
1014	α-Terpinene	tr[b]	0.1±0.0	0.3±0.0	0.9±0.0	0.1±0.0
1022	*p*-Cymene	0.1±0.0	0.1±0.0	---	---	---
1028	Limonene	1.8±0.0	1.3±0.1	5.0±0.0	4.1±0.1	63.1±0.1
1035	(*Z*)-β-Ocimene	0.1±0.0	0.1±0.0	---	---	---
1057	γ-Terpinene	0.1±0.0	0.1±0.0	tr	1.3±0.0	0.2±0.0
1065	*cis*-Sabinene hydrate	---	---	0.1±0.0	0.2±0.0	---
1070	*cis*-Linalool oxide (furanoid)	---	---	tr	0.1±0.0	---
1087	Terpinolene	1.3±0.0	1.1±0.0	0.3±0.0	0.7±0.0	1.0±0.0
1088	2-Nonanone	---	---	tr	---	---
1098	Linalool	---	---	0.2±0.0	0.7±0.0	---
1105	Nonanal	---	---	---	---	tr
1107	*cis*-Rose oxide	---	---	tr	---	---
1118	*cis*-*p*-Menth-2-en-1-ol	---	---	0.1±0.0	0.2±0.0	---
1125	α-Campholenal	0.4±0.0	0.2±0.0	---	---	---
1138	Geijerene	---	---	0.2±0.0	0.1±0.0	tr
1138	*trans*-Pinocarveol	0.3±0.0	0.1±0.0	---	---	---
1141	Camphor	---	---	0.2±0.0	0.2±0.0	tr
1141	*cis*-Verbenol	0.2±0.0	0.1±0.0	---	---	---
1148	*trans*-Verbenol	1.2±0.1	0.4±0.0	---	---	---
1160	*trans*-Pinocamphone	tr	tr	---	---	---
1162	Pinocarvone	0.1±0.0	tr	---	---	---
1169	*p*-Mentha-1,5-dien-8-ol	0.3±0.0	0.2±0.0	---	---	---
1169	Borneol	---	---	0.1±0.0	0.1±0.0	---
1169	Coahuilensol	---	---	1.3±0.3	---	---
1173	*cis*-Pinocamphone	tr	tr	---	---	---
1178	Terpinen-4-ol	0.1±0.0	0.1±0.0	1.1±0.1	2.9±0.1	0.7±0.0
1192	α-Terpineol	0.1±0.0	0.1±0.0	---	0.4±0.1	0.2±0.0
1196	Myrtenal	0.2±0.0	0.1±0.0	---	---	---
1198	Estragole (= Methyl chavicol)	---	---	1.0±0.2	0.6±0.0	tr
1211	Verbenone	1.6±0.0	0.7±0.0	---	---	---
1220	*trans*-Carveol	0.2±0.0	0.1±0.0	---	---	---
1222	Shisofuran	---	---	5.2±0.1	---	---
1242	Carvacrol methyl ether	0.2±0.0	0.1±0.0	---	---	---
1275	Pregeijerene B	---	---	3.8±0.5	4.3±0.5	1.5±0.0
1291	Safrole	0.2±0.0	0.5±0.1	18.8±0.5	22.3±0.5	2.0±0.0
1289	Bornyl acetate	0.1±0.0	0.4±0.0	---	tr	---
1308	α-Cubebene	---	0.5±0.2	---	---	---
1319	(2*E*,4*Z*)-Decadienal	---	---	---	tr	---
1320	Carvacrol	---	---	0.5±0.1	---	---
1323	(2*E*,4*E*)-Decadienal	---	---	tr	tr	---
1331	Anisyl formate	---	---	tr	tr	---
1332	*cis*-Piperitol acetate	---	---	---	tr	---
1341	α-Cubebene	---	---	tr	tr	---
1344	Citronellyl acetate	---	---	---	0.1±0.0	---
1369	Unidentified	---	---	3.1±0.8	---	---
1376	α-Copaene	---	0.1±0.0	---	---	---
1380	Daucene	---	---	---	---	---
1386	*trans*-Myrtanol acetate	tr	0.1±0.0	---	---	---
1395	β-Elemene	---	---	---	0.1±0.0	---
1396	α-Champinene	tr	tr	---	---	---
1402	β-Longipinene	tr	0.1±0.0	---	---	---
1409	Methyl eugenol	0.2±0.0	0.4±0.0	13.8±0.5	11.9±0.0	0.1±0.0
1420	(*E*)-Caryophyllene	3.2±0.0	3.0±0.0	---	---	tr
1432	*cis*-Thujopsene	0.1±0.0	0.7±0.0	---	---	---
1440	γ-Elemene	---	---	---	0.1±0.0	---
1452	*trans*-Muurola-3,5-diene	---	---	---	0.1±0.0	---
1453	α-Humulene	0.3±0.0	0.2±0.0	---	---	---
1457	(*E*)-β-Farnesene	tr	0.1±0.0	---	---	---
1462	*cis*-Thujopsadiene	---	tr	---	---	---
1463	*cis*-Muurola-4(14),5-diene	---	---	tr	tr	---
1473	γ-Muurolene	tr	0.1±0.0	tr	0.1±0.0	---

1473	α-Neocallitropsene	0.2±0.0	0.1±0.0	---	---	---
1473	Dauca-5,8-diene	---	---	---	---	tr
1476	Pinchotene acetate	---	---	1.7±0.0	---	---
1477	*trans*-Cadina-1(6),4-diene	---	---	---	---	tr
1480	Germacrene D	tr	tr	tr	0.7±0.8	tr
1484	β-Selinene	tr	tr	---	---	---
1487	γ-Amorphene	---	---	---	0.1±0.0	---
1489	*trans*-Muurola-4(14),5-diene	---	tr	tr	---	---
1491	Valencene	tr	tr	---	---	---
1493	Viridiflorene	tr	tr	---	---	---
1499	(*E*)-Methyl isoeugenol	1.2±0.0	0.5±0.0	---	---	---
1501	α-Muurolene	---	---	0.2±0.0	0.3±0.0	0.2±0.0
1505	Cuparene	---	0.1±0.0	---	---	---
1511	γ-Cadinene	tr	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0
1508	(*E*,*E*)-α-Farnesene	tr	0.1±0.0	---	---	---
1533	*trans*-Cadina-1,4-diene	---	---	tr	0.1±0.0	tr
1524	δ-Cadinene	0.1±0.0	0.2±0.0	1.0±0.0	1.2±0.0	1.6±0.0
1527	Dauca-4(11),8-diene	---	tr	---	---	---
1540	γ-Cuprenene	---	tr	---	---	---
1538	α-Cadinene	---	tr	0.1±0.0	0.2±0.1	tr
1550	Elemol	0.3±0.0	0.1±0.0	10.6±0.7	13.6±0.7	18.4±0.5
1557	Elemicin	tr	tr	6.8±0.3	7.1±0.5	---
1569	Caryophyllenyl alcohol	tr	tr	---	---	---
1583	Germacrene D-4-ol	tr	0.1±0.0	0.1±0.0	0.2±0.1	---
1589	Caryophyllene oxide	0.6±0.0	0.3±0.0	0.1±0.0	0.1±0.0	---
1599	Widdrol	tr	0.2±0.0	---	---	---
1601	Cedrol	tr	0.2±0.0	---	---	---
1607	Humulene epoxide II	tr	tr	---	---	---
1607	5-*epi*-7-*epi*-α-Eudesmol	---	---	---	0.1±0.0	---
1613	β-Oplopenone	---	---	0.6±0.0	0.2±0.0	---
1616	1,10-di-*epi*-Cubenol	---	---	tr	tr	---
1621	10-*epi*-γ-Eudesmol	---	---	0.1±0.0	0.2±0.0	0.2±0.0
1625	1-*epi*-Cubenol	---	---	0.1±0.0	---	---
1632	γ-Eudesmol	tr	---	1.4±0.1	1.9±0.1	2.5±0.1
1641	τ-Cadinol	tr	tr	0.2±0.1	0.4±0.1	---
1643	τ-Muurolol	tr	0.1±0.0	0.1±0.0	0.5±0.1	0.2±0.0
1645	α-Muurolol (= Torreyol)	---	tr	---	---	0.5±0.0
1650	β-Eudesmol	0.1±0.0	tr	---	---	2.7±0.5
1654	α-Cadinol	0.2±0.0	0.2±0.0	1.5±0.4	1.5±0.0	2.9±0.1
1651	α-Eudesmol	---	---	2.5±0.4	2.9±0.1	---
1666	14-Hydroxy-(*Z*)-caryophyllene	0.1±0.0	0.1±0.0	---	---	---
1677	Bulnesol	---	---	---	tr	---
1688	Botrydiol	---	---	0.3±0.0	0.2±0.0	---
1752	8α,11-Elemodiol	---	---	0.2±0.1	0.1±0.0	---
1791	8α-Acetoxyelemol	---	---	6.0±0.1	4.4±0.0	---
1800	Nootkatone	0.6±0.0	0.3±0.0	---	---	---
1816	Cryptomeridiol	---	---	0.2±0.0	0.1±0.0	---
1886	Oplopanonyl acetate	---	---	tr	tr	---
2055	Abietatriene	tr	0.4±0.0	tr	tr	---
2085	Abieta-7,13-diene	---	---	tr	tr	---
2182	Sandaracopimarinal	0.3±0.0	0.2±0.0	---	---	---
2193	Unidentified diterpenoid	0.6±0.0	0.8±0.0	---	---	---
2221	Unidentified diterpenoid	0.9±0.0	0.9±0.0	---	---	---
2283	Sempervirol	---	---	tr	---	---
2303	4-*epi*-Abietal	---	---	0.1±0.0	0.1±0.0	---
2315	Abieta-7,13-diene-3-one	---	---	tr	tr	---
2319	*trans*-Totarol	---	---	tr	tr	---
2331	*trans*-Ferruginol	0.7±0.0	1.0±0.0	---	---	---
	Total Identified	98.0	97.8	96.5	99.8	100.0
	Compounds Identified	65	73	66	68	33

[a] RI = Retention Index determined with reference to a homologous series of *n*-alkanes on an HP-5ms column. [b] tr = "trace" (< 0.5%).

The chemical compositions of the leaf and bark essential oils of *P. virginiana* are summarized in Table 3. Both the leaf and the bark oils were rich in α-pinene (22.8% and 43.1%, respectively), β-pinene (25.1% and 24.8%, respectively), and β-phellandrene (14.3% and 13.9%, respectively). Many *Pinus* species are rich in α- and β-pinenes [20, 21]. The large concentration of β-phellandrene and the absence of limonene in *P. virginiana* leaf and bark oils was somewhat surprising. The leaf and bark oils of *Pinus taeda* (loblolly pine), a pine that also grows in northern Alabama, was also rich in β-phellandrene and devoid of limonene [22], whereas pines from Italy [21] or Poland [20] had relatively small amounts of β-phellandrene. Interestingly, *P. roxburghii* from Nepal had virtually no α-pinene, β-pinene, nor β-phellandrene in either its leaves or its bark [23].

Table 3: Chemical compositions of *Pinus virginiana* leaf and bark essential oils (average of three measurements ± standard deviations).

RI[a]	Compound	Leaf	Bark
868	(2*E*)-Hexenal	2.0±0.0	---
877	(2*E*)-Hexenol	tr[b]	---
917	Tricyclene	tr	---
941	α-Pinene	22.8±0.4	43.1±0.4
950	Camphene	tr	tr
981	β-Pinene	25.1±0.9	24.8±0.1
993	Myrcene	2.2±0.0	3.3±0.0
1004	α-Phellandrene	0.3±0.0	tr
1006	(3*Z*)-Hexenyl acetate	tr	tr
1015	α-Terpinene	tr	---
1032	β-Phellandrene	14.3±0.2	13.9±0.0
1057	γ-Terpinene	0.1±0.0	---
1074	1-Octanol	tr	---
1083	2-Methoxyethylbenzene	0.7±0.0	---
1088	Terpinolene	0.8±0.0	tr
1103	Linalool	1.5±0.0	tr
1115	*endo*-Fenchol	0.3±0.0	---
1122	*exo*-Fenchol	0.1±0.0	---
1124	*trans*-Rose oxide	0.1±0.0	---
1127	α-Campholenal	0.1±0.0	tr
1140	*trans*-Pinocarveol	---	tr
1141	*trans*-*p*-Menth-2-en-1-ol	0.2±0.0	---
1145	Camphor	---	tr
1148	Camphene hydrate	0.1±0.0	---
1161	Isoborneol	0.1±0.0	---
1168	Borneol	0.2±0.0	tr
1178	Terpinen-4-ol	0.4±0.0	tr
1187	Cryptone	tr	---
1194	α-Terpineol	8.7±0.1	3.7±0.0
1203	*cis*-Piperitol	tr	---
1204	Myrtenol	tr	---
1208	Decanal	0.1±0.0	---
1210	Verbenone	0.1±0.0	tr
1233	Thymol methyl ether	0.1±0.0	tr
1237	Cuminaldehyde	tr	---
1248	(4*Z*)-Decen-1-ol	0.1±0.0	---
1251	Piperitone	0.4±0.0	---
1253	2-Phenylethyl acetate	0.1±0.0	---
1258	(2*E*)-Decenal	0.1±0.0	---
1271	Nonanoic acid	0.1±0.0	---
1287	Bornyl acetate	0.2±0.0	0.4±0.0
1296	2-Undecanone	0.1±0.0	---
1298	Carvacrol	0.1±0.0	tr
1307	Undecanal	tr	---
1318	(2*E*,4*E*)-Decadienal	0.2±0.0	---
1325	(3*Z*)-Hexenyl tiglate	0.1±0.0	---
1334	3-Oxo-*p*-menth-1-en-7-al	tr	---
1350	α-Terpinyl acetate	tr	---
1353	2-Phenylethyl propanoate	tr	---
1376	α-Copaene	tr	---
1384	(3*Z*)-Hexenyl hexanoate	tr	---
1386	Geranyl acetate	tr	---

1389	(3*Z*)-Hexenyl (3*Z*)-hexenoate	tr	---
1393	β-Elemene	tr	---
1398	1-Phenylethyl isobutyrate	0.3±0.0	---
1408	Methyl eugenol	tr	tr
1421	(*E*)-Caryophyllene	0.7±0.0	1.4±0.0
1426	2,5-Dimethoxy-*p*-cymene	tr	---
1436	α-*trans*-Bergamotene	---	tr
1441	α-Guaiene	0.3±0.0	---
1447	2-Phenylethyl butyrate	1.0±0.0	---
1454	α-Humulene	0.2±0.0	0.1±0.0
1459	(*E*)-β-Farnesene	tr	---
1478	γ-Muurolene	0.3±0.0	0.6±0.0
1482	Germacrene-D	0.1±0.0	0.4±0.0
1488	β-Selinene	0.2±0.0	---
1490	2-Phenylethyl 2-methylbutanoate	tr	---
1496	γ-Amorphene	---	tr
1498	Viridiflorene	0.4±0.0	---
1502	α-Muurolene	0.2±0.0	0.4±0.0
1516	γ-Cadinene	0.3±0.0	0.4±0.0
1526	δ-Cadinene	1.1±0.0	1.7±0.0
1535	*trans*-Cadina-1,4-diene	tr	---
1538	α-Cadinene	0.1±0.0	tr
1545	α-Calacorene	tr	tr
1565	β-Calacorene	tr	---
1569	(*E*)-Nerolidol	0.1±0.0	---
1575	(3*Z*)-Hexenyl benzoate	tr	---
1584	Spathulenol	1.3±0.4	---
1587	Caryophyllene oxide	0.5±0.2	---
1588	2-Phenylethyl tiglate	0.2±0.1	---
1595	Salvial-4(14)-en-1-one	---	tr
1596	Viridiflorol	0.1±0.0	---
1598	Ethyl dodecanoate + Cubeban-11-ol	0.1±0.0	---
1606	Rosifoliol	0.1±0.0	---
1611	Humulene epoxide II	tr	---
1616	1,10-di-*epi*-Cubenol	0.1±0.0	tr
1619	Junenol	---	tr
1623	α-Corocalene	---	tr
1629	1-*epi*-Cubenol	0.2±0.0	tr
1643	τ-Muurolol	1.2±0.2	1.5±0.1
1646	τ-Cadinol	1.5±0.0	---
1647	α-Muurolol (= Torreyol)	0.6±0.0	0.3±0.0
1647	2-Phenylethyl hexanoate	0.9±0.2	---
1657	α-Cadinol	4.9±0.1	2.0±0.0
1675	Cadalene	---	0.4±0.0
1728	(2*Z*,6*E*)-Farnesol	0.8±0.0	---
1884	(3*Z*)-Hexenyl cinnamate	0.2±0.0	---
2083	Abieta-7,13-diene	---	tr
2153	Abienol	---	0.2±0.0
2273	Dehydroabietal	---	tr
2305	Abietal	---	0.7±0.1
2340	Methyl dehydroabietate	---	0.2±0.0
2383	Methyl abietate	---	tr
2440	Methyl neoabietate	---	tr
	Total Identified	99.9	99.4
	Compounds Identified	62	20

[a] RI = Retention Index determined with reference to a homologous series of *n*-alkanes on an HP-5ms column. [b] tr = "trace" (< 0.5%).

The essential oils of *Juniperus virginiana* and *Pinus virginiana* were screened for antimicrobial activity against the Gram-positive *Bacillus cereus* and *Staphylococcus aureus*, the Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*, and the pathogenic yeast *Candida albicans* (Table 4). A few of the essential oils of *Juniperus virginiana* and *Pinus virginiana* displayed a marginal level of antibacterial activity against *B. cereus*, *P. aeruginosa*, and *E. coli*. However, the isolated oils displayed very little activity against *S. aureus*, while little to no activity was detected when the oils were screened against *C. albicans*.

Table 4: Antimicrobial activity of *Juniperus virginiana* and *Pinus virginiana* essential oils.

essential oil	MIC (µg/mL)				
	B. cereus	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
J. virginiana					
male bark	625	1250	625	625	1250
female bark	625	1250	625	625	1250
male leaf	625	1250	625	625	625
female leaf	625	625	625	625	625
berries	312.5	1250	625	625	625
P. virginiana					
bark	625	1250	1250	625	2500
leaf	312.5	1250	625	625	2500

Safrole has exhibited antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae*, and *Pityrosporum ovale* [24], but was shown to be inactive against several dermatophytes [25]. Note, however, that safrole has been shown to be hepatotoxic, carcinogenic, and mutagenic [26].
Methyl eugenol has shown antifungal [24, 27] and antibacterial activity [28]. Methyl eugenol also showed quorum sensing inhibitory activity in a *Chromobacterium violaceum* model [29]. Limonene has been shown to be marginally antibacterial and antifungal [30-32] with the (*R*)-(+)-enantiomer more active [33, 34]. Limonene was found, however, to be largely inactive in several antibacterial and antifungal broth dilution assays [35, 36]. α- and β-Pinenes have shown marginal antibacterial activity [37, 38] and antifungal activity [35, 36], and *Pinus* essential oils rich in pinenes have shown antifungal activity [20]. α-Terpineol has also exhibited antibacterial [30] and antifungal activities [35].

4. Conclusions

Although the essential oils of *J. virginiana* did not show appreciable antimicrobial activity in our assays, the high concentrations of limonene in the berries, α-pinene in the bark, and safrole and methyl eugenol in the leaves, may account for the uses of *J. virginiana* in Native American traditional medicine. Similarly, the large quantities of α- and β-pinenes and β-phellandrene in *P. virginiana* likely account for the traditional uses of this plant.

5. Acknowledgments

We are grateful to Dr. Bernhard Vogler for technical assistance with the GC-MS instrumentation and we acknowledge Mr. Bhuwan K. Chherti for assistance with GC-MS interpretation and antimicrobial screening.

6. References

1. Mooney J. The Sacred Formulas of the *Cherokees*. Forgotten Books, London, UK, 2008.
2. Hamel PB, Chiltoskey MU. *Cherokee* Plants and their Uses – A 400 Year History. *Cherokee* Publications, *Cherokee*, North Carolina, USA, 2002.
3. Garrett JT. The *Cherokee* Herbal. Native Plant Medicines from the Four Directions. Bear & Company, Rochester, Vermont, USA, 2003.
4. Radford RE, Ahles HE, Bell CR. Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill, North Carolina, USA, 1968.
5. Brown CL, Kirkman LK. Trees of Georgia and Adjacent States. Timber Press, Portland, Oregon, USA, 1990.
6. Hutton K. A comparative study of the plants used for medicinal purposes by the Creek and Seminole tribes. M.S. Thesis, University of South Florida, 2010.
7. Nolan JM. The roots of tradition: Social ecology, cultural geography, and medicinal plant knowledge in the Ozark-Ouachita Highlands. Journal of Ethnobiology 1998; 18(2):249-269.
8. Payne KW, Wittwer R, Anderson S, Eisenbraun E. Use of a modified Abderhalden apparatus for comparing laboratory and industrial methods of isolating eastern redcedar essential oil. Forest Products Journal 1999; 49(7):90-92.
9. Eller FJ, Taylor SL. Pressurized fluids for extraction of cedarwood oil from *Juniperus virginiana*. Journal of Agricultural and Food Chemistry 2004; 52:2335-2338.
10. Semen E, Hiziroglu S. Production, yield and derivatives of volatile oils from eastern redcedar (*Juniperus virginiana* L.) American Journal of Environmental Sciences 2005; 1(2):133-138.
11. Adams RP. Cedar wood oil – Analyses and properties. In Linskens HF, Jackson JF (Eds), Essential Oils and Waxes, Springer, Berlin, Heidelberg, 1991, 159-173.
12. Dunford NT, Hiziroglu S, Holcomb R. Effect of age on the distribution of oil in eastern redcedar tree segments. Bioresource Technolog 2007; 98:2636-2640.
13. Setzer WN. Leaf and bark essential oil compositions of *Bursera simaruba* from Monteverde, Costa Rica. American Journal of Essential Oils and Natural Products 2014; 1(3):34-36.
14. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, Edn 4, Allured Publishing, Carol Stream, Illinois, 2007.
15. Woods KE, Chhetri BK, Jones CD, Goel N, Setzer WN. Bioactivities and compositions of *Betula nigra* essential oils. Journal of Medicinally Active Plants 2013; 2(1):1-9.
16. Von RE. Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochemical Systematics and Ecology 1975; 2:131-167.
17. Setzer WN, Whitaker KW, Lawton RO. A chemical ecological study of the components of the essential oil of eastern red cedar (*Juniperus virginiana*) from three habitats in Huntsville, Alabama. Castanea 1992; 57:209-213.
18. Gawde AJ, Cantrell CL, Zheljazkov VD. Dual extraction of essential oil and podophyllotoxin from *Juniperus virginiana*. Industrial Crops and Products 2009; 30:276-280.
19. Hădărugă NG, Branic AG, Hădărugă DI, Gruia A, Pleșa C, Costescu C *et al*. Comparative study of *Juniperus virginiana* essential oils: TLC and GC analysis. Journal of Planar Chromatography 2011; 24:130-135.
20. Krauze-Baranowska M, Mardarowicz M, Wiwart M, Pobłocka L, Dynowska M. Antifungal activity of the essential oils from some species of the genus *Pinus*.

Zeitschrift für Naturforschung, 2002; 57c:478-482.
21. Macchioni F, Cioni PL, Flamini G, Morelli I, Maccioni S, Ansaldi M. Chemical composition of essential oils from needles, branches and cones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy. Flavour and Fragrance Journal 2003; 18139-143.
22. Kennedy JE, Davé PC, Harbin LN, Setzer WN. Allelopathic potential of *Sassafras albidum* and *Pinus taeda* essential oils. Allelopathy Journal 2011; 27(1):111-122.
23. Satyal P, Paudel P, Raut J, Deo A, Dosoky NS, Setzer WN. Volatile constituents of *Pinus roxburghii* from Nepal. Pharmacognosy Research 2013; 5(1):43-48.
24. Kubo I, Muroi H, Himejima M. Combination effects of antifungal nagilactones against *Candida albicans* and two other fungi with phenylpropanoids. Journal of Natural Products 1993; 56:220-226.
25. Zacchino SA, López SN, Pezzenati GD, Furlán RL, Santecchia CB, Muñoz L *et al. In vitro* evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes. Journal of Natural Products 1999; 62:1353-1357.
26. Ionnides C, Delaforge M, Parke DV. Safrole: Its metabolism, carcinogenicity and interactions with cytochrome P-450. Food and Cosmetics Toxicology 1981; 19:657-666.
27. Voda K, Boh B, Vrtačnik M, Pohleven F. Effect of the antifungal activity of oxygenated aromatic essential oil compounds on the white-rot *Trametes versicolor* and the brown-rot *Coniophora puteana*. International Biodeterioration & Biodegradation 2003; 51:51-59.
28. Joshi RK. Chemical composition, *in vitro* antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum*, *O. sanctum* and their major constituents. Indian Journal of Pharmaceutical Sciences 2013; 75(4):457-462.
29. Packiavathy IASV, Agilandeswari P, Musthafa KS, Pandian SK, Ravi AV. Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. Food Research International 2012; 45:85-92.
30. Kubo I, Muroi H, Kubo A. Naturally occurring antiacne agents. Journal of Natural Products 1994; 57(1):9-17.
31. Filipowicz N, Kamiński, Kurlenda J, Asztemborska M, Ochocka JR. Antibacterial and antifungal activity of juniper berry oil and its selected components. Phytotherapy Research 2003; 17:227-231.
32. Chee HY, Kim H, Lee MH. *In vitro* antifungal activity of limonene against *Trichophyton rubrum*. Mycobiology 2009; 37(3):243-246.
33. Lis-Balchin M, Ochocka RJ, Deans S, Asztemborska M, Hart S. Bioactivity of the enantiomers of limonene. Medical Science Research 1996; 24(5):309-310.
34. Aggarwal KK, Khanuja SPS, Ahmad A, Kumar TRS, Gupta VK, Kumar S. Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa*. Flavour and Fragrance Journal 2002; 17:59-63.
35. Hammer KA, Carson CF, Riley TV. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. Journal of Applied Microbiology 2003; 95:853-860.
36. Schmidt JM, Noletto JA, Vogler B, Setzer WN. Abaco bush medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. Journal of Herbs, Spices & Medicinal Plants 2006; 12(3):43-65.
37. Mourey A, Canillac N. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control 2002; 13:289-292.
38. Palazzo MC, Wright HL, Agius BR, Wright BS, Moriarity DM, Haber WA *et al.* Chemical compositions and biological activities of leaf essential oils of six species of Annonaceae from Monteverde, Costa Rica. Records of Natural Products 2009; 3(3):153-160.