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Characterization of Fetterbush *Lyonia lucida* Liquid Extractions

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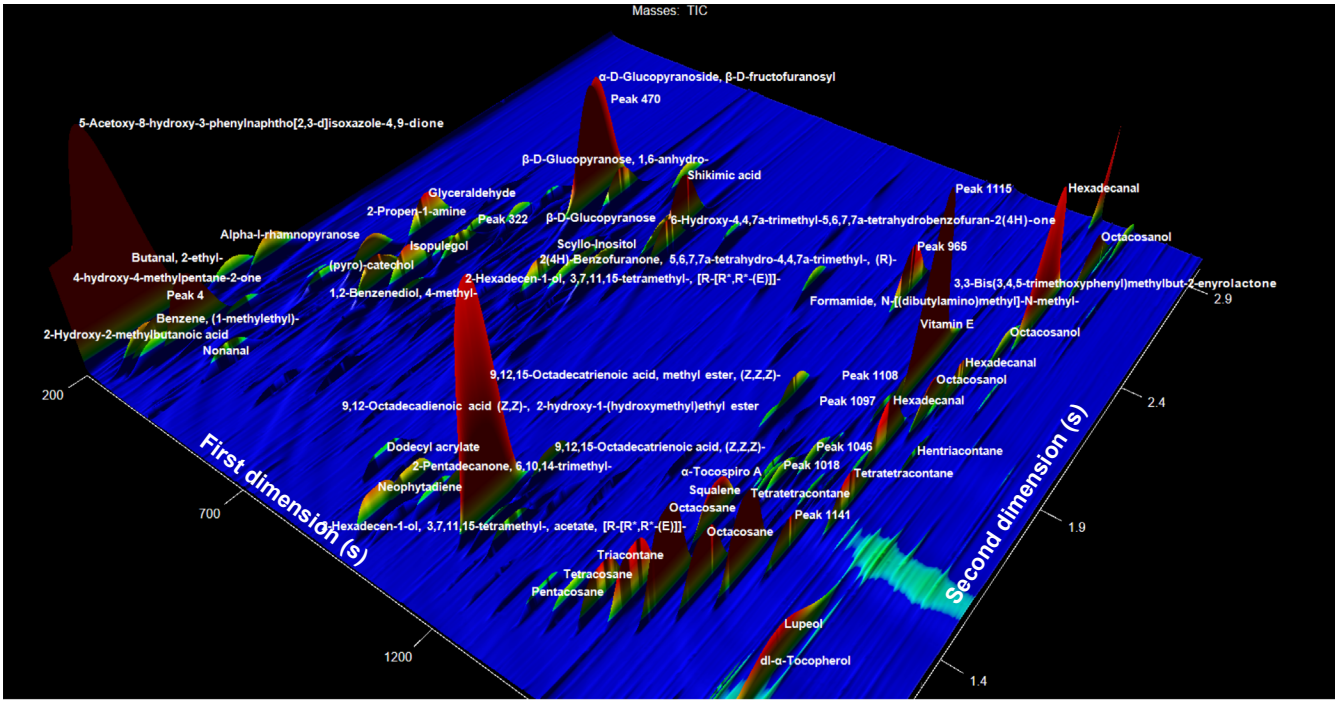

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Abstract

Fetterbush (*Lyonia lucida*) grows in the southern states of the United States. The chemical components in fetterbush are highly concentrated with phenolic compounds. These compounds contribute to the toxicity of the plant and could possibly be igniters for wildfire. In this study, liquid extractions were obtained from fresh Fetterbush samples. Two-dimensional gas chromatography and time of flight mass spectrometry (GC×GC-TOFMS 4D) were used to characterize the chemical compositions in the liquid extractions. GC×GC technology has existed for more than 30 years. This technique opens the peak capacity in which trace and hidden chemicals can be identified. GC×GC detected 1,899 and 916 chemicals from the solvent and bead bug extractions, respectively. There were 72 chemicals shared by both methods identified with similarity match >900.

Keywords: GC×GC; extractives; bushes, *Lyonia lucida*; liquid extraction

Contents

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Characterization of Fetterbush *Lyonia lucida* Liquid Extractions

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Introduction

Fetterbush (*Lyonia lucida*) is a shrub native to the southern United States. This bush, along with many other species, is being investigated to determine its contribution in the severe wildfires that take place every year. Pyrolysis modeling is important to understanding wildland fire. Amini and others (2019) used slow pyrolysis to identify more than 200 compounds in tar from dead and live vegetation. Countless forest fires occur yearly and sometimes grow uncontrollably and rapidly. The chemicals from the various bushes found on the floor of the forest are known for contributing fuel to these fires. Fetterbush is one of the vegetations being used for pyrolysis modeling. Fetterbush is highly toxic, possibly fatal if eaten, and produces allelic compounds that deter plant growth in its vicinity (Fisher 1987). The poison parts of the bush are the leaves and nectar from the flowers. The chemicals from this plant are of extreme interest for medicinal, herbicidal, and potential fuel source purposes. In this study, the primary chemicals of fetterbush leaves were extracted by two solvent methods. Liquid extractions are known for separating chemicals into various fractionation using different solvents. (Moore and others 2015a, 2015b, 2017a, 2017b). Both methods used solvents, but one method was shaken and the other method used steel beads to remove the extracts from the leaves. Comprehensive two-dimensional gas chromatography and time of flight mass spectrometry (GC×GC TOFMS) were used to identify the isolated extractives from both extraction methods. This comparison determined which method isolated the most chemicals from the leaves. The chemical fingerprint from the GC×GC chromatograms will assist in identifying chemical groups that could possibly be hazardous fuel sources in wildland fires.

Experimental Methods

Materials

Fetterbush plants were purchased from a nursery. The moisture content of the plants was kept at 30%. Solvents were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Tetracosane 99% is the calibration standard and was purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Solvent Extraction

Fetterbush leaves were extracted using solvent extraction (SE). The leaves were picked and frozen until ready to be extracted. The leaves were slightly thawed and weighed and then placed in a 250-mL Erlenmeyer flask with 100 mL of solvent solution. The first solvent solution (fb-mle) was made of acetone and water (90:10), and the second solvent solution (fb-c2) was made of hexane and isopropyl alcohol (50:50). In both solvent extractions, the solvent solutions were filtered, and fresh solvent was poured over the leaves every 24 hours. The leaves in the solution were shaken (orbital shaker) for a total of 48 hours. Solvent solutions were evaporated using a rotary evaporator, and then the extracts were transferred to preweighed vials, sealed, and stored in the freezer.

Bead Bug Extraction

Bead Bug (BB) (Benchmark Scientific, Edison, New Jersey, USA) was also used for extraction of fetterbush leaves. The BB uses a bead mill vial containing steel beads. The BB vials were filled with 1:1 hexane/isopropanol solution (fb-c2) and were centrifuged for 2 min at 400 rpm. After this cycle, the vials were placed in a desktop centrifuge at 12,000 rpm for 1 to 2 min. The supernatant was decanted into a 10-mL glass syringe and filtered with a tarred nylon 0.45-µm luer lock into a 50-mL flask. This was repeated until the leaves were a brownish tan color and all the green was gone from them. For the last extraction, a hand vortex was used for 50 seconds to remove any remaining extractives in the leaves. Four replicates were performed using 9:1 acetone and water (fb-mle) and were collected in different 50-mL volumetric flasks. At this point in the procedure, the tissue was completely pulverized.

Table 1—Gas chromatography and two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC-TOFMS) (Pegasus BT 4D) instrument parameters

Gas chromatograph agilent 7890B with LECO dual stage quad jet modulator and LPAL 3 autosampler	
Injection	1 uL, split 20–150:1 at 250 °C
Carrier gas	He at 1.4 mL/min
Primary column:	Rxi-5 ms, 30-m by 0.25-mm i.d. by 0.25-µm coating (Restek, Bellefonte, PA, USA)
Secondary column	Rxi-17SilMS, 0.60-m by 0.25-mm by 0.25-µm coating (Restek, Bellefonte, PA, USA)
Oven programming	Primary column starting temperature 50 °C, ramped 10 °C/min to 320 °C, held 5 min. Secondary column temperature parallel ramp offset by +5 °C (total run time 32 min).
Modulation	3–5 s with temperature maintained +15 °C relative to second oven
Transfer line	280 °C
Mass spectrometer LECO Pegasus BT 4D	
Ion source temperature	250 °C
Mass range	50–650 m/z
Acquisition rate	150 spectra/s (2D)

Instrumentation Operations

Table 1 shows the GC×GC instrument parameters. The oven programming temperature for the secondary column was 5 °C higher than the primary column. The transfer line and injection temperatures remain the same for every run. The split ratio varied based on the concentration of the complex mixture. The modulation temperature was 15 °C higher than the secondary oven. The retention time for the second dimension was less than 5 s for each sample.

Results and Discussion

Two solvent extractions were performed for each method (SE and BB), but only the first extraction from each method was used for the GC×GC analysis. The first extraction from the SE method was the acetone extraction (fb-mle), and the first extraction from the BB method was the hexane extraction (fb-c2). The extractions were run in triplicates. NIST and Wiley libraries were used to establish spectral similarity between the chemicals identified in the fetterbush complex extract mixtures. The minimum similarity for matches was 700. The number of chemicals identified from the library databases were 1,899 and 916 chemicals in the acetone (fb-mle) and hexane (fb-c2) extracts, respectively.

Table 2 lists the number of chemicals detected at various similarity matches. There were 252 and 96 unknown chemicals detected for the fb-mle and fb-c2 extracts, respectively.

Table 2—Number of chemicals detected by two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOFMS) and identified by NIST and Wiley libraries

Solvent solution	Total >700	Peaks unknown	Peaks known	>800 known	>800 known[a]
fb-mle (SE method)	1,899	252	1,647	798	245
fb-c2 (BB method)	916	96	820	530	104

[a]Number of known chemicals after removing shared and isomers.

Chemicals with isomers and duplicates caused by high concentrations were removed from the list. The modified number of chemicals detected was 245 in fb-mle and 104 in fb-c2 with greater than 800 similarity matches. Seventy-two chemicals were shared between both extracts. These were not included in the total for each extract.

The lists of chemicals in Tables 3 and 4 are not duplicates and are specific to their solvent and extraction method. The chemicals listed are with spectral matches greater than 900. The percentage peak area was calculated using the sum of all chemicals with matches greater than 800 similarity. Several ketones and furanones were identified from the fb-mle extracts. These chemicals are known for various flavors and scents. Shikimic acid is used in antiviral chemicals such as that in Tamiflu (Genentech, Inc., South San Francisco, California, USA). The chemicals in Tables 3 and 4 did not yield any significantly high percentage peak areas with spectral matches at 900 similarity. However, there were chemicals below the 900 spectral matches with high percentage peak areas such as 1,2,3,4,5,6-cyclohexanehexol 11.97% and 1-ethyl-5-methylcyclopentene 9.71% in the fb-mle extracts and 2-hexanol 14.19% and (+)lactic acid 11.63% in the fb-c2 extracts.

GC×GC analysis identified 72 chemicals shared between both solvent methods. Sugars and phenolic compounds had high percentage peak areas. Tables 5 and 6 show chemicals with greater than 900 similarity matches. Fewer chemicals were identified in the fb-c2 extracts. This could have been caused by the concentration of the extracts. Fb-mle chemicals with high percentage peak area were β-D-glucopyranose; 1,6- anhydro- 23.8%; (pyro)-catechol 12.6%; and 5-hydroxymethylfurfural 7.65%. Glucose 10.9% was high, but it was below the 900 similarity match threshold. Sugars were highly concentrated in the fb-mle extracts.

Figures 1 and 2 show different ways to visually demonstrate the GC×GC chromatograms of the fb-mle (SE method) extract. Figure 1 is a surface plot, and Figure 2 is a contour plot. The retention time of the chemicals eluted in the first dimension is based on volatility, and in the second dimension, it is according to polarity. Any ripple in the blue surface is an indication of a chemical being detected. The black dots on the contour plot are chemicals detected. The peaks that are most intense or highly concentrated are red.

Table 3—Two-dimensional gas chromatography-time of flight mass spectometry (GC×GC-TOFMS) list of compounds specifically identified in fetterbush extracts (fb-mle, SE method) >900 similarity

Number	Chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	4-Hydroxy-4-methylpentane-2-one	$C_6H_{12}O_2$	4.359	203.001 0.989	960
2	Maleic anhydride	$C_4H_2O_3$	0.012	209.004 1.459	931
3	2-Furanmethanol	$C_5H_6O_2$	1.822	212.005 1.080	944
4	5-O-Methoxymethylbacillariolide I	$C_{22}H_{32}O_4$	0.391	215.006 0.972	918
5	Malonic acid	$C_3H_4O_4$	0.018	230.012 0.489	904
6	Acetic acid	$C_2H_4O_2$	0.276	233.014 0.605	909
7	2,3-Butanedione	$C_4H_6O_2$	0.148	242.017 0.316	962
8	1-Hydroxy-3-penten-2-one	$C_5H_8O_2$	1.489	251.021 1.198	951
9	2(5H)-Furanone	$C_4H_4O_2$	0.777	257.024 1.735	962
10	1,2-Propadiene-1,3-dione	C_3O_2	0.141	266.027 1.390	928
11	5-Methyl furfural	$C_6H_6O_2$	3.144	296.04 1.383	916
12	3-Hydroxy-2(5H)-furanone	$C_4H_4O_3$	0.088	299.041 1.550	927
13	Methyl 6-formyl-hept-6-eneoate	$C_9H_{14}O_3$	0.007	317.048 0.841	943
14	β-Myrcene	$C_{10}H_{16}$	0.470	317.048 0.908	941
15	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	0.981	317.048 1.838	905
16	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	0.102	320.05 1.502	909
17	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	0.298	329.053 1.219	933
18	Anhydromevalonolactone	$C_6H_8O_2$	0.008	362.067 2.112	938
19	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	$C_{10}H_{16}$	0.355	368.07 0.957	937
20	3.,22(S),29-Trihydroxy-22,25-epoxylanost-9(11)-ene	$C_{30}H_{50}O_4$	0.071	371.071 1.456	940
21	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	1.044	374.072 1.366	914
22	5-Hydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_3$	8.548	404.084 1.532	916
23	Benzene, 1-methyl-4-(1-methylethenyl)-	$C_{10}H_{12}$	0.041	407.086 1.159	900
24	Undecane	$C_{11}H_{24}$	0.124	410.087 0.818	932
25	Benzeneethanol	$C_8H_{10}O$	0.294	428.094 1.468	932
26	3-Allyloxypentane	$C_8H_{16}O$	0.059	443.101 1.354	902
27	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	1.730	452.104 1.512	924
28	2,2-Dimethyl-4-oxidanyl-hexan-3-one	$C_8H_{16}O_2$	0.025	470.112 0.639	906
29	5-(Hydroxymethyl)dihydro-2(3H)-furanone	$C_5H_8O_3$	0.090	491.121 2.142	911
30	FURAN	C_4H_4O	0.080	506.127 1.862	929
31	3-Butenal-2-one	$C_4H_4O_2$	0.011	512.129 2.218	929
32	3-Cyclohexene-1-acetaldehyde, α,4-dimethyl-	$C_{10}H_{16}O$	0.368	518.132 1.285	904
33	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	0.098	524.134 1.102	901
34	Cyclopentanol, 2-ethenyl-2-methoxy-, trans-	$C_8H_{14}O_2$	0.029	524.134 1.712	999
35	2-Decenal, (Z)-	$C_{10}H_{18}O$	0.046	551.145 1.145	910
36	Benzoic acid, 2-(acetyloxy)-	$C_9H_8O_4$	0.128	581.158 1.348	909
37	Ketene	C_2H_2O	0.025	599.165 0.551	941
38	(5S)-5-Ethyl-2-oxolanone	$C_6H_{10}O_2$	0.005	611.17 1.110	921
39	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6-methyl-	$C_7H_{14}O_5$	0.461	626.176 1.641	933
40	1,6-Anhydro-β-d-talopyranose	$C_6H_{10}O_5$	0.749	638.181 2.087	904
41	(3-Propyl-2-oxiranyl)methanol	$C_6H_{12}O_2$	0.136	653.188 1.734	918
42	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	0.509	683.2 1.813	920
43	2-Heptanoylfuran	$C_{11}H_{16}O_2$	0.021	695.205 0.631	907
44	2-Methoxy-4-(n-propyl)phenol	$C_{10}H_{14}O_2$	0.026	713.212 1.788	905
45	p-Hydroxybenzoic Acid	$C_7H_6O_3$	0.524	737.222 1.761	903
46	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	10.719	740.224 0.538	942
47	α-Selinene	$C_{15}H_{24}$	0.010	746.226 1.149	912

Table 3—Two-dimensional gas chromatography-time of flight mass spectometry (GC×GC-TOFMS) list of compounds specifically identified in fetterbush extracts (fb-mle, SE method) >900 similarity—con.

Number	Chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
48	3,5-Diethylhex-5-en-3-ol	$C_{10}H_{20}O$	0.029	749.227 0.701	904
49	Oct-7-en-1-ol	$C_8H_{16}O$	0.018	770.236 0.594	906
50	Benzaldehyde, 3,4-dihydroxy-	$C_7H_6O_3$	0.160	797.247 1.914	904
51	3,4-Dihydroxyphenyl-2-propanone	$C_9H_{10}O_3$	0.161	863.275 1.963	904
52	Dodecyl acrylate	$C_{15}H_{28}O_2$	0.044	872.278 1.064	921
53	D-Allose	$C_6H_{12}O_6$	3.675	920.298 1.162	910
54	Shikimic acid	$C_7H_{10}O_5$	2.453	926.301 2.120	934
55	Phytol	$C_{20}H_{40}O$	0.619	1,124.38 1.091	938
56	Docosane	$C_{22}H_{46}$	0.046	1,169.4 0.939	938
57	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.025	1,253.44 1.312	923
58	3-Eicosene, (E)-	$C_{20}H_{40}$	0.007	1,313.46 1.179	918
59	Pentacosane	$C_{25}H_{52}$	0.156	1,361.48 1.030	947
60	Hentriacontane	$C_{31}H_{64}$	0.153	1,490.53 1.159	940
61	Methyl 5-(4-methoxyphenyl)-2,2,4,4-tetramethyl-3,5-dioxopentanoate	$C_{17}H_{22}O_5$	0.060	1,577.57 0.607	912
62	Octacosanol	$C_{28}H_{58}O$	0.035	1,733.63 2.780	901
63	(3aS,6S,6aS)-6,8,8-Trimethyl-3,3a,4,5,6,6a,7,9-octahydroazuleno[4,5-c]furan-1-one	$C_{15}H_{22}O_2$	0.012	1,760.65 1.789	904
64	Friedelan-3-one	$C_{30}H_{50}O$	0.180	1,790.66 2.671	907

Table 4—Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of compounds specifically identified in fetterbush extracts (fb-c2 BB method) >900 similarity

Number	Chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	4,5-Bis(3',4'-methylenedioxyphenyl)benzo[15]crown-5	$C_{34}H_{44}O_{13}$	0.36	212.005 0.083	978
2	2-Propanone 1-(acetyloxy)-	$C_5H_8O_3$	0.27	218.007 1.199	939
3	Isopropyl alcohol	C_3H_8O	0.73	224.01 0.383	900
4	2,5-Hexanedione	$C_6H_{10}O_2$	0.26	266.027 1.361	950
5	9-Oxa-cis(1,8),cis(2,7),trans(7,8)-tricyclo[6.3.0,0(2,7)] undeca-3,5,10-triene	$C_{14}H_{14}O$	0.58	299.041 1.170	922
6	1,2,3-Propanetriol	$C_3H_8O_3$	0.06	314.047 1.366	905
7	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	0.09	452.104 1.511	937
8	Undecane 2-methyl-	$C_{12}H_{26}$	0.07	467.111 0.822	938
9	Cyclobutanone	C_4H_6O	1.18	473.113 2.427	949
10	Undecane 2,6-dimethyl-	$C_{13}H_{28}$	0.26	509.128 0.823	907
11	Benzaldehyde 3-hydroxy-4-methoxy-	$C_8H_8O_3$	0.13	665.193 1.860	931
12	Mequinol	$C_7H_8O_2$	0.09	758.231 0.703	900
13	Scyllo-inositol	$C_6H_{12}O_6$	2.47	818.256 2.117	937
14	Pentadecane 2,6,10-trimethyl-	$C_{18}H_{38}$	0.04	842.266 0.859	902
15	Neophytadiene	$C_{20}H_{38}$	0.37	965.317 0.939	931
16	1,2,4,5-Tetrakis(4-phenylphenyl)benzene	$C_{54}H_{38}$	0.49	1,889.7 0.382	992

Table 5—Two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOFMS) list of shared compounds identified in fetterbush extracts (fb-mle SE method) >900 similarity

Number	Shared chemicals identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	Benzene (1-methylethyl)-	C_9H_{12}	0.517	266.027 0.980	907
2	Glycerin	$C_3H_8O_3$	0.273	296.04 1.459	933
3	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.958	308.045 1.128	918
4	Phenol	C_6H_6O	2.480	308.045 1.252	931
5	(Octahydro-7a-methylbenzofuran-6-yl) Formate	$C_{10}H_{14}O_3$	0.306	350.062 0.572	909
6	Limonene	$C_{10}H_{16}$	0.575	353.063 0.963	915
7	Benzenemethanol	C_7H_8O	1.474	356.065 1.431	939
8	Nonanal	$C_9H_{18}O$	0.062	416.089 1.064	916
9	(Pyro)-catechol	$C_6H_6O_2$	12.630	497.123 1.481	924
10	Benzofuran 2,3-dihydro-	C_8H_8O	2.904	515.13 1.471	912
11	5-Hydroxymethylfurfural	$C_6H_6O_3$	7.652	521.133 1.895	924
12	1,2-Benzenediol 4-methyl-	$C_7H_8O_2$	1.000	572.154 1.515	901
13	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.629	599.165 1.499	924
14	2,6-Octadienoic acid 3,7-dimethyl- (E)-	$C_{10}H_{16}O_2$	4.962	623.175 1.220	912
15	Phenol 2-methoxy-3-(2-propenyl)- or guaiacol 6-allyl-	$C_{10}H_{12}O2$	2.789	632.179 1.446	910
16	Benzaldehyde 4-hydroxy-	$C_7H_6O_2$	0.065	632.179 1.760	908
17	1,4-Benzenediol 2-methoxy-	$C_7H_8O_3$	0.283	662.191 1.812	900
18	Hydroxychavicol	$C_9H_{10}O_2$	1.655	719.215 1.573	907
19	β-D-Glucopyranose 1,6-anhydro-	$C_6H_{10}O_5$	22.217	725.217 2.245	927
20	β-D-Glucopyranoside methyl	$C_7H_{14}O_6$	5.290	791.245 1.916	944
21	Benzenepropanol 4-hydroxy-3-methoxy-	$C_{10}H_{14}O_3$	0.788	848.268 1.763	912
22	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.070	992.328 0.952	905
23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.162	1,037.35 1.110	923
24	Eicosane	$C_{20}H_{42}$	0.046	1,115.38 0.926	916
25	Octadecanoic acid	$C_{18}H_{36}O_2$	0.035	1,148.39 1.141	903
26	(20R,22R,24S)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-cyclo-5-α-cholestane	$C_{28}H_{48}O_5$	0.214	1,151.39 0.492	905
27	Triacontane	$C_{30}H_{62}$	0.124	1,220.42 0.954	917
28	(1,2-Dihydroxyethyl)oxirane	$C_4H_8O_3$	0.069	1,322.46 0.504	967
29	Arbutin	$C_{12}H_{16}O_7$	0.562	1,337.47 0.104	904
30	Heptacosane	$C_{27}H_{56}$	0.154	1,448.52 1.107	920
31	Squalene	$C_{30}H_{50}$	0.104	1,463.52 1.393	910
32	Vitamin E	$C_{29}H_{50}O_2$	0.309	1,589.58 2.220	910
33	Hexadecanal	$C_{16}H_{32}O$	0.053	1,622.59 1.830	900
34	1-Docosene	$C_{22}H_{44}$	0.042	1,685.62 2.337	907
35	7-Hydroxy-7-phenyl-3,9-diisopropyl-2,10-dioxadispiro[3.3.3.1]dodecan-1,11-dione	$C_{22}H_{28}O_5$	0.599	1,781.65 0.140	968
36	Furan 2,3-dihydro-	C_4H_6O	0.709	1,898.7 0.084	999

Table 6—Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of shared compounds identified of fetterbush extracts (fb-c2, BB method) >900 similarity

Number	Shared chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	(20R,22R,24S)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-cyclo-5-α-cholestane	$C_{28}H_{48}O_5$	19.380	203.001 0.994	957
2	7H-Isobenzofuro[4,5-b][1,4]benzodioxepin-11-carboxaldehyde, 1-(acetyloxy)-5-[(acetyloxy) methyl]-1,3-dihydro-4,10-dihydroxy-8-	$C_{22}H_{16}O_{12}$	0.181	227.011 0.098	973
3	2-Propanone	C_3H_6O	1.089	260.025 0.251	975
4	Benzene, (1-methylethyl)-	C_9H_{12}	0.425	266.027 0.986	931
5	2-Propenal, 3-(2-furanyl)-	$C_7H_6O_2$	1.361	269.029 0.580	956
6	Benzenemethanol	C_7H_8O	0.494	356.065 1.429	926
7	Nonanal	$C_9H_{18}O$	0.073	416.089 1.062	943
8	(Pyro)-catechol	$C_6H_6O_2$	2.461	494.122 1.501	925
9	(Octahydro-7a-methylbenzofuran-6-yl) Formate	$C_{10}H_{14}O_3$	3.941	548.144 0.541	915
10	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	1.216	596.164 1.523	925
11	2,6-Octadienoic acid, 3,7-dimethyl-, (E)-	$C_{10}H_{16}O_2$	6.048	623.175 1.211	937
12	Benzaldehyde, 4-hydroxy-	$C_7H_6O_2$	0.050	629.178 1.787	927
13	Tetradecane	$C_{14}H_{30}$	0.447	671.195 0.846	900
14	Phenol, 2-methoxy-4-(1-propenyl)- or eugenol	$C_{10}H_{12}O_2$	0.328	704.209 1.525	919
15	Hydroxychavicol	$C_9H_{10}O_2$	1.494	719.215 1.577	910
16	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	23.794	719.215 2.263	931
17	β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	0.408	779.24 1.983	924
18	Benzenepropanol, 4-hydroxy-3-methoxy-	$C_{10}H_{14}O_3$	0.710	848.268 1.773	909
19	p-Coumaric acid	$C_9H_8O_3$	0.168	926.301 2.016	911
20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.126	992.328 0.954	915
21	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.233	1,037.35 1.114	923
22	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	0.433	1,124.38 1.094	934
23	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	0.206	1,139.39 1.295	900
24	1-Docosene	$C_{22}H_{44}$	0.207	1,313.46 1.178	933
25	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	$C_{30}H_{50}$	0.743	1,463.52 1.393	933
26	1-Cyclohexene-1-carboxaldehyde, 2-(3,3-dimethyl-1-butenyl)-, (E)-	$C_{13}H_{20}O$	0.138	1,517.55 0.618	933
27	γ-Tocopherol	$C_{28}H_{48}O_2$	0.529	1,559.56 2.056	901
28	β-Sitosterol	$C_{29}H_{50}O$	0.553	1,673.61 0.107	927
29	7-Hydroxy-7-phenyl-3,9-diisopropyl-2,10-dioxadispiro[3.3.3.1]dodecan-1,11-dione	$C_{22}H_{28}O_5$	0.665	1,694.62 0.349	970

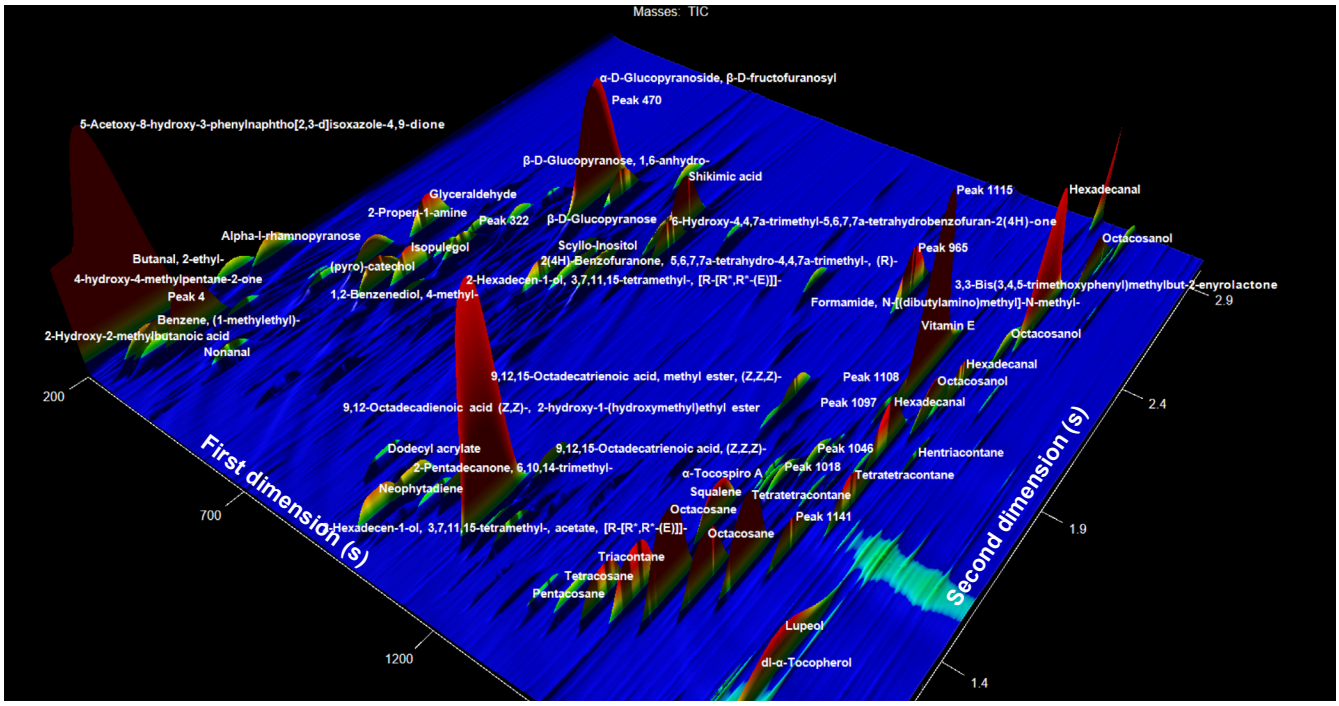

Figure 1. Two-dimensional gas chromatography (GC×GC) chromatogram surface plot for fetterbush extracts (fb-mle, SE method).

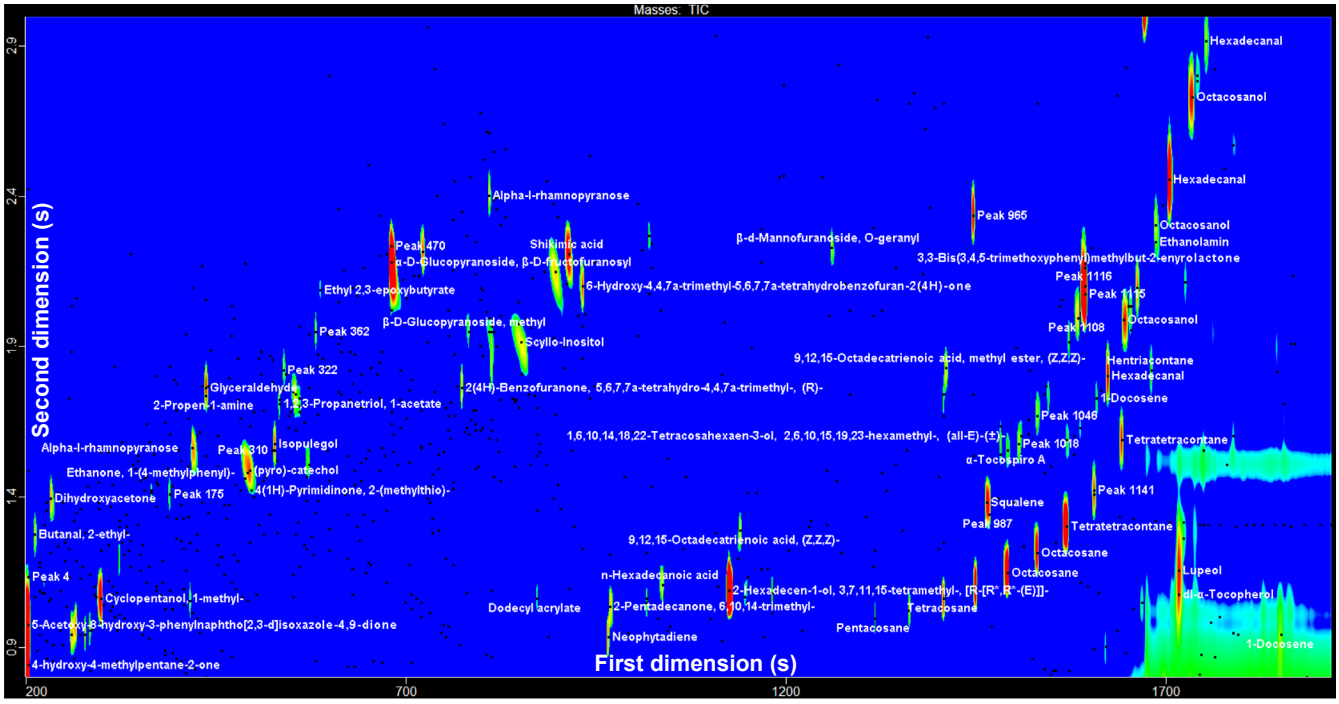

Figure 2. Two-dimensional gas chromatography (GC×GC) chromatogram contour plot for fetterbush extracts (fb-mle, SE method).

A GC×GC chromatogram is known for displaying different regions for various classes of chemicals found in complex mixtures. This is called molecular chemical mapping (MCM). The formatting notice in these chromatograms is similar to the periodic table. The periodic table is grouped according to chemical families and physical properties. GC×GC chromatograms can be used to create a fingerprint for various complex mixtures. This is one of the best ways to visually and chemically identify unknown chemicals in complex mixtures.

On the bottom left side of the chromatograms were long chain alcohols, aldehydes, and alkenes. In this region, there were two unknown peaks at 1018 and 1046. Because of the position of the chemicals and the spectrum of the unknown, we used the known chemicals around the unknown chemical with its spectrum to establish the chemical name. Each unknown has a spectrum that can be used to determine the structure.

The chemical composition of fb-mle extracts contained several sugars, and they were extremely concentrated. In the middle top of the chromatogram, there were several sugars visually detected. There is also an unknown peak (470) detected in this region. Visual assessment of the position of the peak on the chromatogram could assist with identifying the chemical name from the mass spectrum.

Identifying regions of classes of chemical (RCC) on the chromatograms is important when characterizing the chemicals found in complex mixtures. Because of the resolving power and the visual view of MCM on a GC×GC chromatogram, we can identify chemicals in extracts. This technique will help with future characterization of chemical contributors to wildfires in our forests.

Conclusion

The GC×GC detected 1,899 chemicals from the first extraction using the SE method (fb-mle) and 912 from the first extraction using the BB method (fb-c2). These results could be based on the polarities of the different solvents used in the extractions. However, there were 72 shared chemicals identified in both extractions. It can be assumed but not concluded that there are some similarities in the polarity of both solvent systems.

We believe the concentration played a role in the number of chemicals detected in the fb-c2 extract. There were several unknown chemicals detected. The spectra of these chemicals will be important in characterizing them. There is not much literature on the chemical components of fetterbush. Shikimic acid was identified in fetterbush. This chemical is typically found in sweetgum trees. Fetterbush could be another source for isolating chemicals with antiviral properties. Further characterization of the second extractions from each method will be investigated. Which chemicals or chemical groups could be the contributing igniters in wildfires is still under review. GC×GC TOFMS is a great analytical technique to further the investigation on identifying these chemicals. This study is also the first to identify chemicals in fetterbush leaves using GC×GC TOFMS.

Acknowledgments

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