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Investigation of Inkberry *Ilex glabra* L. Gray (Aquifoliaceae) Solvent Fractions using Two-Dimensional Gas Chromatography Technology

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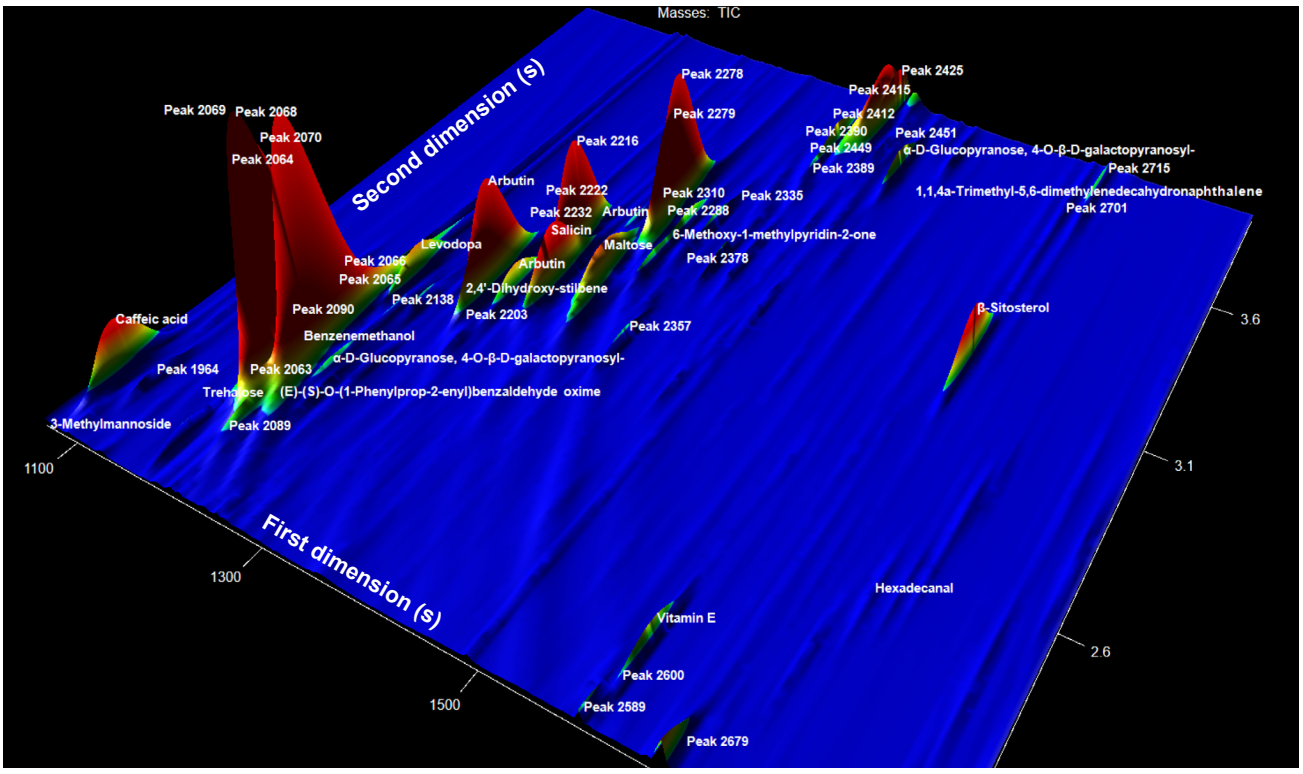

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Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) is an important technique used to investigate complex mixtures with pharmaceutical potential. GC×GC expands the peak capacity of the separation, which increases the resolution for more targeted characterization of analytes of interest and trace chemicals. Traditional gas chromatography–mass spectrometry is used to investigate extracts; however, when the mixture is complex, there is a need for higher resolution power to identify the extracts profile completely and discover new extracts of interest. In this study, inkberry chemical compounds were characterized using GC×GC. Inkberry was chosen because of the lack of literature on its chemical components. Two solvent solutions were used to isolate chemicals from inkberry leaves, acetone and hexane. From the acetone extracts, 2,986 chemicals were identified and 1,653 of them were unknowns. From the hexane extracts, 1,726 chemicals were identified and 698 were unknowns. NIST and Wiley libraries were used for identification.

Keywords: GC×GC, extractives, bushes, fractions, solvent extraction

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Introduction

Comprehensive two-dimensional gas chromatography (GC×GC) consisted of two tandem columns with different chemical stationary phases. These phases separate complex mixtures with a thermal modulator to divert one effluent from a one-dimensional to a two-dimensional column. This technique has been available for more than 30 years and was first reported by Lui and Phillips (1991). The two different stationary phases increase the peak capacity in which co-eluting chemicals are easy to identify in complex mixtures (Lui and Phillips). GC×GC was chosen for this investigation because of the complexity and similarities of the chemicals in inkberry.

Inkberry (*Ilex glabra* (L.) Gray) is an ornamental plant of the Holly family (Aquifoliaceae) and is also known as gallberry. The name inkberry gives reference to the fruit that is produced by the shrubs, which was once used to produce ink. It is an evergreen native to the coastal plain of eastern North America. Inkberry leaves are used for an herbal tea called Appalachian. The name gallberry was derived from the galls of oaks, which can be used to make black ink. In addition to its application in ink production, this plant is resistant to pollutants in urban areas and does not have any serious problems with insects or diseases. Bees and bird are drawn to it as a source for food.

The holly plant family has been studied for their phytochemicals. Jaiswal and others (2014) detected and characterized 32 phenolic compounds by liquid chromatography tandem mass spectrometry. They reported for the first time full characterization of 3,4-dihydroxybenzoyl glucosides; 3,4-dihydroxybenzyl glucosides; 4-hydroxybenzoyl glucosides; chlorogenic acid glucosides; and vanillic acid glucosides. Polyphenols are the largest and most complex group of phytochemicals found in most human and animal diets. Biomass such as plants and wood are constructed with multiple chemical structures of phenolic derivatives. The phenolic compounds can range from very toxic to nontoxic. These properties are of interest to scientific research. The free radicals created from reducing phenolic compounds give rise to antioxidant activity, which has many health benefits. The similarity of these phenolic compounds will cause co-eluting and decrease the similarity match between chemicals in the sample and the library (Moore and others 2015a, 2015b, 2017a, 2017b, 2017c). The complexity and similarity between the chemicals require more resolving power to identify trace chemicals in the samples.

Comprehensive two-dimensional gas chromatography time of flight (GC×GC-TOF) was chosen for this investigation because of the complexity and similarities of the chemicals found in inkberry extractives. Acetone and hexane extracts were characterized and categorized based on the molecular chemical mapping on the chromatogram. The chromatograms of these solvent fractions were categorized based on their functionality. Chemical mapping provided a fingerprint of inkberry extractives in both solvent fractions.

Experimental

Materials

Inkberry plants were purchased at a local nursery. These plants were maintained with moisture content above 30%. Solvents were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Tetracosane 99% was used for the calibration standard and was purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Extraction Procedure

Inkberry leaves were extracted by two solvent solutions. Leaves were removed from the freezer and slightly thawed. They were weighed and placed in a 250-mL Erlenmeyer flask with 100 mL of acetone and water (90:10) solvent solution. The second extraction was performed with isopropyl alcohol and hexane (50:50). The solvent solution extractions were filtered, filled with fresh solvent every 24 hours, and shaken for a total of 48 hours. Solvent

Table 1—Two-dimensional gas chromatography (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 to 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)

solutions were evaporated using rotary evaporation, transferred to preweighed vials, sealed, and stored in the freezer.

Instrumentation Operations

The GC×GC instrument parameters are given in Table 1. The instrument operation was the same for every run. The split ratio varied based on the concentration of the samples. The modulation time depended on the retention of the chemicals from the second column. The samples were spiked with a known concentration of tetracosane, and the instrument was calibrated with various concentrations of tetracosane.

Results and Discussion

The spectral similarities of the inkberry chemicals were determined by National Institute of Standards and Technology (NIST) and Wiley libraries. The spectral similarity match minimum was 700. The numbers of chemicals identified from the library databases were 2,986 and 1,726 in the acetone (iba) and hexane (ibh) extracts, respectively. Table 2 shows the number of chemicals detected at various similarity matches. There were 1,653 and 698 unknown chemicals detected for iba and ibh extracts, respectively.

Several chemicals were removed because of duplication. Isomers were also identified and removed. The modified numbers of chemicals detected with spectral matches greater than 800 were 183 and 141 for iba and ibh extracts, respectively. The chemicals shared by both extractions were not included in the total.

Table 2—Number of chemicals detected by two-dimensional gas chromatography and identified by NIST and Wiley libraries

Extract	Total >700	Peaks unknown	Peaks known	>800 known	>800 known[a]
iba	2,986	1,653	1,333	659	183
ibh	1,726	698	1,029	558	141

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

Tables 3 and 4 list chemicals specifically identified with spectral matches greater than 900 similarity for each solvent extraction. All duplicates were removed from the list. The percentage peak area was calculated using the sum of all chemicals with matches greater than 800 similarity. Table 3 shows that acetone extracts had a high percentage of sugars. This is within expectation because acetone was mixed with 10% water. Also, 25.8% of the peak area in the mixture was detected for 3,4-dihydroxy-benzoic acid. This is not seen in Table 3 because it did not meet the spectral match greater than 900. Table 4 shows that (pyro)-catechol had a high area percentage detected in the extract mixture. Also, 4.80% of the peak area in the mixture was detected for 3,7,11,15,2-hexadecen-1-ol. There were significant amounts of alkanes in low concentration in the hexane extracts.

There were 83 chemicals shared by both solvent mixtures. Tables 5 and 6 list the shared chemicals with spectral matches greater than 900 similarity. Uvaol was detected at ~4% in both solvent systems. This chemical is a triterpenoid and is considered a secondary metabolite (Braca and others 2003). Glucose was detected at 12.5% in the acetone extracts. Other sugars such as 1,6 anhydro-glucopyranose and muco-inositol were detected at ~4% and ~5%, respectively (not shown). Benzyl β-d-glucoside and vanillic acid were detected. Jaiswal and others (2014) detected the same chemicals using liquid chromatography tandem mass. Benzyl b-d glucoside is a plant metabolite that has anti-inflammatory activity in cellular assays. It is known for contributing to antidiabetic effects.

Figures 1 and 2 show different ways to visually demonstrate the GC×GC chromatograms of the acetone 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 the second dimension is according to polarity. Any ripples in the blue surface are an indication of chemicals being detected. The black dots on the contour plot are chemicals detected. The peaks that are most intense or highly concentrated are red.

Complex mixtures have many chemicals with similar chemical properties, which causes co-elution and peak

Table 3—List of compounds specifically identified in inkberry acetone extracts with two-dimensional gas chromatography >900 similarity

No.	Name iba	Formula	Peak area (%)	Retention time (s)	Similarity
1	Formic acid	CH_2O_2	0.011	203.999 0.599	905
2	3-Furaldehyde	$C_5H_4O_2$	0.476	203.999 0.898	908
3	(20R,22R,24S)-20,22,24,25-Tetrahydroxy-6β-methoxy-3α,5-cyclo-5-α-cholestane	$C_{28}H_{48}O_5$	4.152	203.999 0.996	935
4	Furfural	$C_5H_4O_2$	1.233	203.999 1.133	942
5	Glyceraldehyde	$C_3H_6O_3$	0.184	207.999 1.131	938
6	2-Furanmethanol	$C_5H_6O_2$	1.489	211.998 1.087	921
7	Maleic anhydride	$C_4H_2O_3$	0.157	211.998 1.453	916
8	1-(Acetyloxy)-2-propanone	$C_5H_8O_3$	0.137	219.997 1.191	913
9	1-Hydroxy-3-penten-2-one	$C_5H_8O_2$	0.045	251.993 1.198	924
10	Ethanol, 2-(2-methoxyethoxy)-	$C_5H_{12}O_3$	0.051	275.989 1.189	943
11	5-Methyl furfural	$C_6H_6O_2$	0.298	299.986 1.369	916
12	2-Furanmethanol, α-3-butenyltetrahydro-α,2-dimethyl-, (R*,S*)-	$C_{11}H_{20}O_2$	0.007	315.984 0.073	947
13	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	0.143	327.982 1.228	924
14	4-Hydroxy-5,6-di-hydro-(2H)-pyran-2-one	$C_5H_6O_3$	0.329	327.982 1.522	920
15	5-Methylhex-3-yn-2-yl acetate	$C_9H_{14}O_2$	0.024	331.981 1.520	917
16	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	0.015	395.972 1.828	917
17	(1R,2R,5S,7S)-7-Ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]-octan-2-ol	$C_9H_{16}O_3$	0.009	411.97 1.167	939
18	4H-Pyran-4-one, 2,3-dihydro-3,5-	$C_6H_8O_4$	2.00	451.965 1.514	928
19	Benzoic acid	$C_7H_6O_2$	1.651	463.963 1.385	916
20	Benzeneacetic acid	$C_8H_8O_2$	0.120	535.953 1.475	945
21	1-Methoxy-1-(4-methylphenyl)propan-2-	$C_{11}H_{22}O_2$	0.018	535.953 1.652	963
22	2-Methyl-2-phenyl-malonaldehyde	$C_{10}H_{10}O_2$	0.008	535.953 1.798	943
23	7H-Isobenzofuro[4,5-b][1,4]benzodioxepin-11-carboxaldehyde, 1-(acetyloxy)-5-[(acetyloxy)methyl]-1,3-	$C_{22}H_{16}O_{12}$	0.050	583.946 1.423	969
24	[2-13C]Cyclohexane-1,3-dione	$C_6H_8O_2$	0.014	607.943 2.034	994
25	Vanillic acid	$C_8H_8O_4$	1.117	783.918 1.785	914
26	Scyllo-Inositol	$C_6H_{12}O_6$	5.951	907.901 1.706	925
27	Sedoheptulosan	$C_7H_{12}O_6$	4.798	911.9 2.316	912
28	(1,2-Dihydroxyethyl)oxirane	$C_4H_8O_3$	0.158	943.896 1.531	933
29	Hexadecanoic acid	$C_{16}H_{32}O_2$	0.067	1035.88 1.115	918
30	Pentacos-1-ene	$C_{25}H_{50}$	0.007	1307.84 1.028	935
31	Triacontane	$C_{30}H_{62}$	0.144	1447.82 1.109	939
32	[(2S,3S)-3-Methyl-2-oxiranyl]methanol	$C_4H_8O_2$	0.060	1555.81 0.463	906
33	1-Hexacosene	$C_{26}H_{52}$	0.021	1559.81 1.353	911
34	2-Methyl-2-[4'-(methoxymethyleneoxy)phenyl]-(1,3)-	$C_{12}H_{16}O_4$	0.065	1567.81 0.463	976
35	Hexadecanal	$C_{16}H_{32}O$	0.039	1623.8 1.828	904
36	((4aR,8aS)-3-Methoxy-3-phenyl-octahydro-isochromen-1-yl)-phenyl-methanone	$C_{23}H_{26}O_3$	0.005	1659.8 2.199	944

Table 4—List of compounds specifically identified in inkberry hexane extracts with two-dimensional gas chromatography >900 similarity

No.	Name ibh	Formula	Peak area (%)	Retention time (s)	Similarity
1	Methyltartronic acid	$C_4H_6O_5$	0.110	218.007 0.942	953
2	(5R)-5-(Hydroxymethyl)-2-oxolanone	$C_5H_8O_3$	0.017	236.015 1.407	905
3	Heptanal	$C_7H_{14}O$	0.044	245.019 0.952	900
4	2-Propenal, 3-(2-furanyl)-	$C_7H_6O_2$	0.315	248.02 2.113	925
5	2,3-Octanedione	$C_8H_{14}O_2$	0.025	311.046 1.009	922
6	5-Hepten-2-one, 6-methyl-	$C_8H_{14}O$	0.304	314.047 1.075	911
7	Cyclohexene, 1-methyl-4-(1-	$C_{10}H_{18}$	0.022	347.061 0.918	903
8	Limonene	$C_{10}H_{16}$	0.140	353.063 0.958	925
9	2,5-Furandione, 3,4-dimethyl-	$C_6H_6O_3$	0.047	362.067 1.578	957
10	Cis/trans-2-Cyclohexyl-5-methyltetrahydrofuran	$C_{11}H_{20}O$	0.005	380.075 2.148	902
11	Furyl hydroxymethyl ketone	$C_6H_6O_3$	0.013	401.083 1.557	924
12	(Z,Z)-3,6-Nonadienal	$C_9H_{14}O$	0.056	410.087 1.159	933
13	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	0.246	452.104 1.501	932
14	2-Hydroxysuccinaldehyde	$C_4H_6O_3$	0.197	458.107 2.192	914
15	(Pyro)-catechol	$C_6H_6O_2$	7.147	494.122 1.491	910
16	Decanal	$C_{10}H_{20}O$	0.020	503.126 1.076	923
17	Benzofuran, 2,3-dihydro-	C_8H_8O	1.060	515.13 1.468	926
18	1,2-Benzenediol, 4-methyl-	$C_7H_8O_2$	0.456	572.154 1.513	927
19	Tridecane	$C_{13}H_{28}$	0.042	581.158 0.855	926
20	5,9-Undecadien-2-one, 6,10-dimethyl-,	$C_{13}H_{22}O$	0.238	704.209 1.178	941
21	1-Dodecanol	$C_{12}H_{26}O$	0.053	719.215 1.053	930
22	Hexadecane	$C_{16}H_{34}$	0.036	737.222 0.871	920
23	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	0.618	773.237 1.762	938
24	2-Propen-1-ol, 2-methyl-, acetate	$C_6H_{10}O_2$	0.084	812.253 2.598	953
25	Myristic acid	$C_{14}H_{28}O_2$	0.155	914.296 1.093	941
26	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.600	968.318 1.034	923
27	Nonadecane	$C_{19}H_{40}$	0.027	1001.33 0.897	901
28	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	$C_{18}H_{30}O$	0.032	1016.34 1.213	901
29	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.442	1037.35 1.109	932
30	2-Hexadecen-1-ol, 3,7,11,15-	$C_{20}H_{40}O$	4.809	1124.38 1.092	940
31	Heneicosane	$C_{21}H_{44}$	0.054	1169.4 0.937	905
32	Tetradecanal	$C_{14}H_{28}O$	0.019	1184.41 1.111	902
33	Tricosane	$C_{23}H_{48}$	1.488	1406.5 1.059	945
34	Squalene	$C_{30}H_{50}$	3.833	1463.52 1.388	936
35	Hexadecanal	$C_{16}H_{32}O$	0.076	1547.56 1.513	913
36	Octacosane, 2-methyl-	$C_{29}H_{60}$	0.028	1553.56 1.260	905
37	4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-	$C_{15}H_{10}O_6$	0.007	1604.58 2.264	901
38	Stigmasterol	$C_{29}H_{48}O$	0.066	1646.6 2.862	907

Table 5—List of shared compounds identified in inkberry acetone extracts with two-dimensional gas chromatography >900 similarity

No.	Name iba (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
1	2(5H)-Furanone	$C_4H_4O_2$	0.641	259.992 1.718	944
2	2-Heptenal, (E)-	$C_7H_{12}O$	0.038	287.988 1.087	903
3	3-Hydroxy-2(5H)-furanone	$C_4H_4O_3$	0.163	299.986 1.551	918
4	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.600	307.985 1.131	941
5	Phenol	C_6H_6O	2.042	311.984 1.236	938
6	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	0.254	319.983 1.822	924
7	Benzenemethanol	C_7H_8O	1.481	359.978 1.413	935
8	(Furan-2-yl)methanol	$C_5H_6O_2$	0.017	383.974 0.772	918
9	5-Allyltetrahydrofuran-2-one	$C_7H_{10}O_2$	0.021	383.974 2.119	904
10	Undecane	$C_{11}H_{24}$	0.059	411.97 0.819	935
11	Nonanal	$C_9H_{18}O$	0.038	415.97 1.068	926
12	5-(Hydroxymethyl)dihydrofuran-2(3H)-one	$C_5H_8O_3$	0.268	491.959 2.141	923
13	2-Decenal, (Z)-	$C_{10}H_{18}O$	0.036	551.951 1.145	936
14	Salicylic acid	$C_7H_6O_3$	0.173	579.947 1.358	915
15	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.497	599.944 1.499	920
16	Isopropyl alcohol	C_3H_8O	19.601	891.903 0.507	911
17	Glucose	$C_6H_{12}O_6$	12.557	939.896 1.888	903
18	Hexadecanoic acid	$C_{16}H_{32}O_2$	0.067	1035.88 1.115	918
19	Phytol	$C_{20}H_{40}O$	0.499	1127.87 1.083	948
20	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	0.039	1139.87 1.289	908
21	Tetracosane	$C_{24}H_{50}$	0.042	1271.85 0.971	962
22	Pentacosane	$C_{25}H_{52}$	0.259	1407.83 1.061	945
23	Octacosane	$C_{28}H_{58}$	1.248	1491.82 1.157	945
24	1,3-Dioxane, 4-ethenyl-2,2-dimethyl-6-phenyl-, cis-	$C_{14}H_{18}O_2$	0.016	1563.81 2.069	917
25	Tetratetracontane	$C_{44}H_{90}$	0.441	1567.81 1.314	951
26	Vitamin E	$C_{29}H_{50}O_2$	0.161	1591.8 2.208	910
27	Heptacosane	$C_{27}H_{56}$	0.026	1639.8 1.604	929
28	Octacosanol	$C_{28}H_{58}O$	0.144	1647.8 2.007	913
29	β-Sitosterol	$C_{29}H_{50}O$	0.105	1671.79 3.126	930
30	Furan, 2,3-dihydro-	C_4H_6O	0.698	1871.77 0.335	903
31	Olean-12-ene-3,28-diol, (3β)-	$C_{30}H_{50}O_2$	0.695	1875.76 0.030	914
32	Uvaol	$C_{30}H_{50}O_2$	4.810	1911.76 1.227	904

Table 6—List of shared compounds identified in inkberry hexane extracts with two-dimensional gas chromatography >900 similarity

No.	Name ibh (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
1	4-Hydroxy-4-methylpentane-2-one	$C_6H_{12}O_2$	4.87	203.001 0.640	922
2	2(5H)-Furanone	$C_4H_4O_2$	0.22	257.024 1.725	911
3	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	0.30	263.026 1.322	926
4	2-Heptenal, (E)-	$C_7H_{12}O$	0.08	290.037 1.073	936
5	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.44	308.045 1.119	903
6	Benzenemethanol	C_7H_8O	0.10	359.066 1.403	918
7	Undecane	$C_{11}H_{24}$	0.33	410.087 0.818	946
8	Anhydro - sugar	$C_5H_8O_4$	0.09	413.088 1.915	911
9	Nonanal	$C_9H_{18}O$	0.33	416.089 1.059	962
10	5-Allyltetrahydrofuran-2-one	$C_7H_{10}O_2$	0.01	437.098 2.055	930
11	Butanedioic acid	$C_4H_6O_4$	0.57	458.107 1.337	922
12	5-(Hydroxymethyl)dihydrofuran-2(3H)-one	$C_5H_8O_3$	0.05	491.121 2.142	921
13	Nonanoic acid	$C_9H_{18}O_2$	0.20	551.145 1.069	941
14	2-Decenal, (Z)-	$C_{10}H_{18}O$	0.08	551.145 1.141	942
15	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	0.07	626.176 1.717	901
16	Benzaldehyde, 4-hydroxy-	$C_7H_6O_2$	0.08	629.178 1.774	911
17	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	$C_{13}H_{18}$	0.08	638.181 1.258	900
18	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	0.02	668.194 1.823	923
19	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	3.70	722.216 2.226	928
20	p-Hydroxybenzoic acid	$C_7H_6O_3$	0.597	737.222 1.754	916
21	4-Hydroxy-3-methoxy-benzoic acid	$C_8H_8O_4$	0.19	782.241 1.761	904
22	Muco-inositol	$C_6H_{12}O_6$	5.03	863.275 1.839	927
23	p-Coumaric acid	$C_9H_8O_3$	0.13	926.301 2.000	901
24	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.442	1037.35 1.109	932
25	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,	$C_{20}H_{40}O$	4.809	1124.38 1.092	940
26	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	0.17	1136.39 1.191	917
27	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	0.47	1139.39 1.288	925
28	Octadecanoic acid	$C_{18}H_{36}O_2$	0.09	1148.39 1.137	916
29	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.13	1253.44 1.308	943
30	Tetracosane	$C_{24}H_{50}$	0.33	1268.44 0.975	959
31	Pentacosane	$C_{25}H_{52}$	0.29	1361.48 1.027	951
32	Octacosane	$C_{28}H_{58}$	1.90	1448.52 1.103	941
33	Nonacos-1-ene	$C_{29}H_{58}$	0.04	1481.53 1.191	922
34	Tetratetracontane	$C_{44}H_{90}$	4.31	1529.55 1.229	937
35	1,3-Dioxane, 4-ethenyl-2,2-dimethyl-6-phenyl-, cis-	$C_{14}H_{18}O_2$	0.06	1562.56 2.071	924
36	Octacosanol	$C_{28}H_{58}O$	2.88	1643.6 2.011	931
37	Furan, 2,3-dihydro-	C_4H_6O	0.13	1784.66 0.612	999
38	Uvaol	$C_{30}H_{50}O_2$	4.70	1910.71 0.166	900

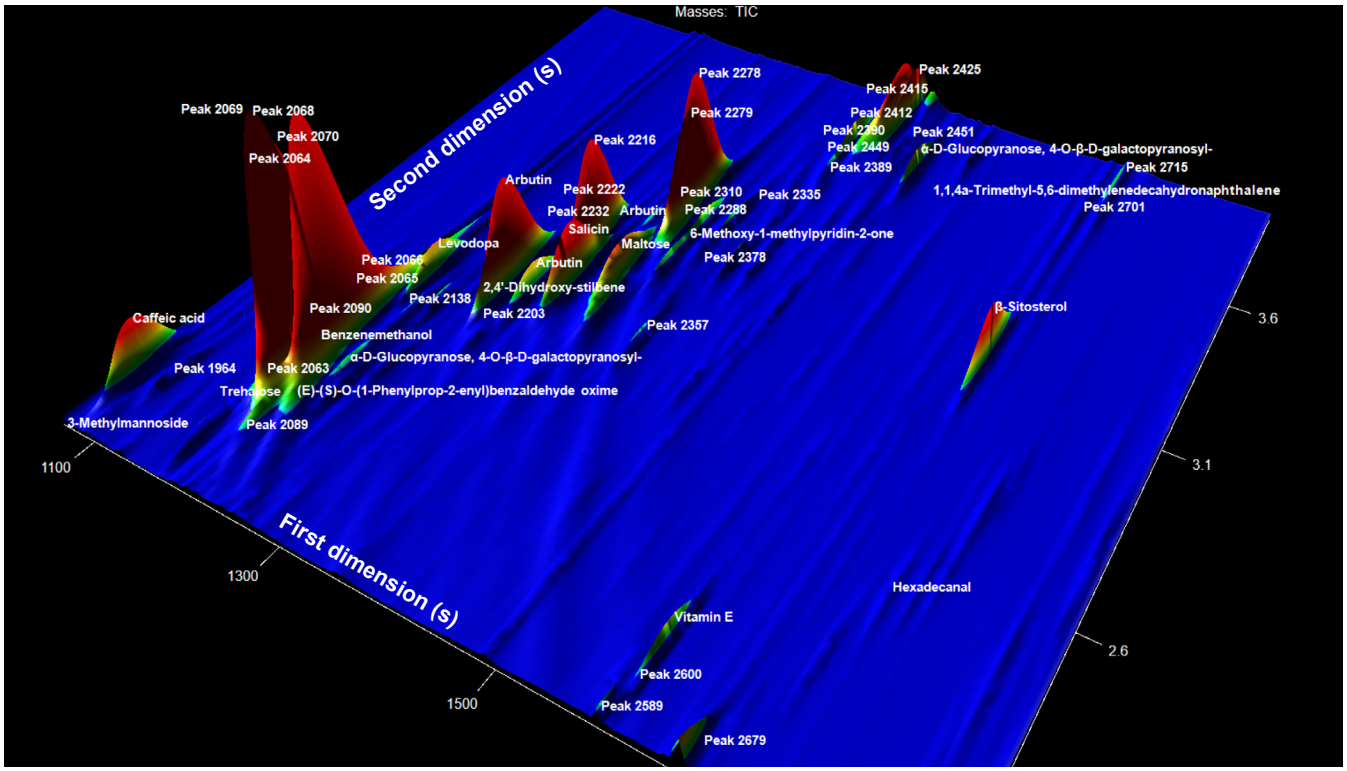

Figure 1. GC×GC chromatogram surface plot of inkberry acetone extracts.

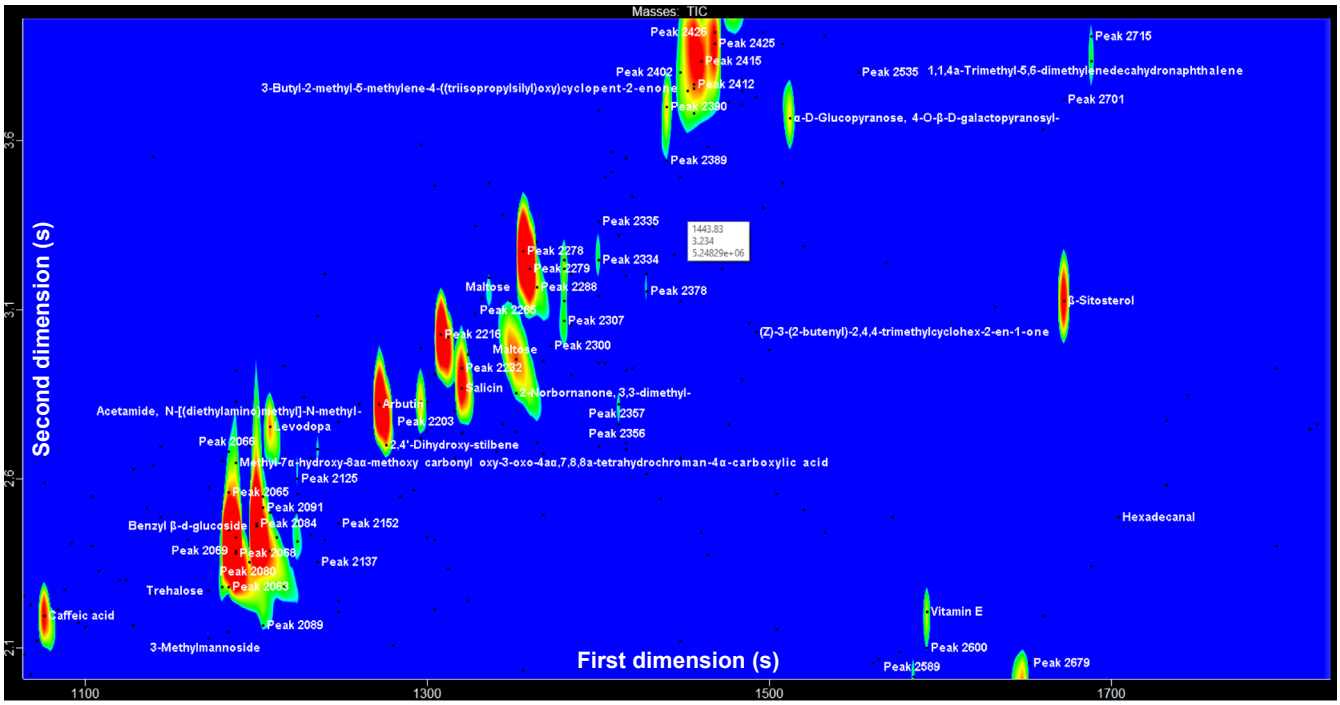

Figure 2. GC×GC chromatogram contour plot of inkberry acetone extracts.

broadening on the one-dimensional GC chromatogram. The 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). MCM is similar to the periodic table in that different classes or families of chemicals are in the same area on the chromatogram. 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.

Characterization of inkberry extracts is extremely complex. There were thousands of unknown chemicals detected in the acetone extracts. The zoom-in chromatogram of the hexane extracts showed a visible region of phenolic compounds eluted between 500 and 700 seconds in the first dimension (not shown). Extracts from different solvents will assist with characterizing the complex mixture of chemicals from inkberry. In this mixture, there were more concentrated aromatics than long chain hydrocarbon compounds. The long chain hydrocarbon compounds were detected, but they were not concentrated. The sterols and naphthalene regions were identified. The aromatic chemicals were highly concentrated in the inkberry extracts.

There were several unknown peaks detected in this mixture. However, if there are known chemicals on the chemical map, this will assist with understanding the chemical structure from the unknown peak mass spectrum.

GC×GC chromatograms are important when characterizing complex mixtures. These chemicals are sectioned into regions with similar classes of chemicals. The fingerprint for complex mixtures is established with GC×GC chromatograms. The resolving power and visual view of MCM allows identification of unknown chemicals in complex mixtures. This technique is changing the way we investigate complex mixtures.

Conclusions

Two inkberry extracts with different solvents were characterized using GC×GC. There were 2,986 and 1,726 chemicals detected in the iba and ibh extracts, respectively. There were 1,653 and 698 unknown chemicals extracted, respectively. There were several classes of chemicals identified and characterized by the GC×GC. The chromatogram showed different regions where similar chemicals were eluted. Some of these chemicals were concentrated, and some were in trace amounts. Furanones, sugars, phenolics, alkanes, and fatty acid were some of the classes of chemicals identified in these solvent mixtures. Benzyl β-d-glucoside and vanillic acid were identified and were mentioned in the literature. One of the best techniques for characterizing inkberry extracts is GC×GC. In the chromatograms, we were able to visually identify functional group trends. This visual trend is important to identifying unknown chemicals along with their spectra. Future work will include determining the chemical names for the unknown chemicals identified and testing the extracts for biological activity.

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