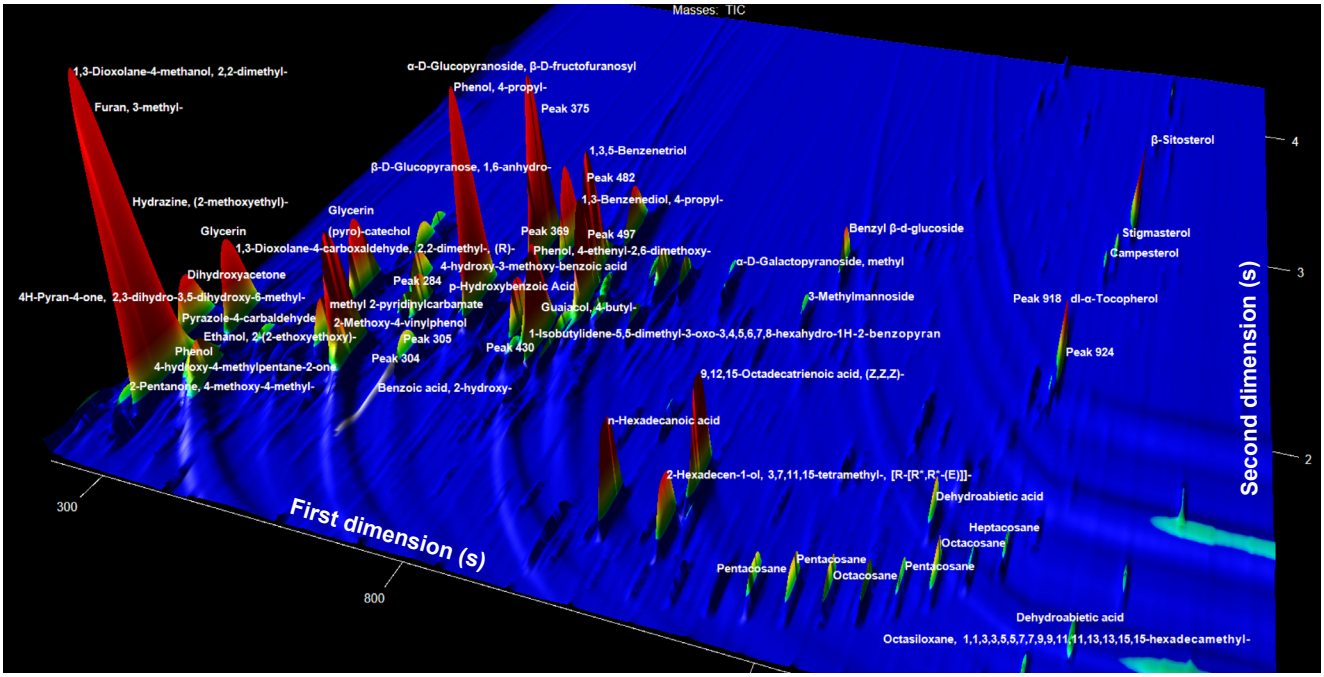
United States Department of Agriculture

Two-Dimensional Gas Chromatography Characterization of Saw Palmetto *Serenoa repens* Chemical Composition

Roderquita K. Moore
Doreen Mann



Forest Service

Forest Products Laboratory

Research Note FPL–RN–0387

September 2020

Abstract

Comprehensive two-dimensional gas chromatography (GC×GC) is an important technique used to investigate potential medicinal mixtures. The pharmaceutical industry is a trillion-dollar industry, and 25% of this value comes from plant-derived chemicals. Saw Palmetto (*Serenoa repens*) berries are known for their medicinal properties. This medicinal alternative aids in treatment of health problems such as asthma, benign prostatic hypertrophy, colds, migraines, prostate cancer, and sore throats. Cancer is a disease where abnormal cells rapidly divide and destroy body tissue. In 2018, an estimated 1.7 million new cases of cancer were diagnosed in the United States and 609,640 people will die from the disease. This disease affects people in different ways. The most common type of cancer is breast cancer, followed by lung and prostate cancer. In 2019, about 174,650 new cases of prostate cancer were diagnosed, and about 31,620 ended in death. One in nine men will be diagnosed with prostate cancer, and it mainly affects older men and African-American men. In this study, the chemical composition of saw palmetto leaves was characterized using GC×GC-time of flight mass spectrometry. Extractions were taken with two different solvents. Similar chemicals were identified in both solvent mixtures. Fatty acids and sterols compounds used to relieve urological disorders were identified in the saw palmetto leaves.

Keywords: GC×GC, extractives, bushes, *Serenoa repens*, extraction

September 2020

Moore, Roderquita K; Mann, Doreen. 2020. Two-dimensional gas chromatography characterization of saw palmetto *Serenoa repens* chemical composition. Research Note FPL-RN-0387. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 10 p.

A limited number of free copies of this publication are available to the public from the Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53726-2398. This publication is also available online at www.fpl.fs.fed.us. Laboratory publications are sent to hundreds of libraries in the United States and elsewhere.

The Forest Products Laboratory is maintained in cooperation with the University of Wisconsin.

Contents

Two-Dimensional Gas Chromatography Characterization of Saw Palmetto *Serenoa repens* Chemical Composition

Roderquita K. Moore, Research Chemist
Doreen Mann, Physical Science Technician
USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, USA

Introduction

Our ancestors used natural products and home remedies to treat sickness and diseases. Saw palmetto (*Serenoa repens*) berry extracts are known for their alternative use for treating urological disorders. Willetts and others (2003) tested the effects of *Serenoa repens* extracts in a double-blind placebo-controlled random trial. In that study, it was concluded that there was improvement in benign prostatic hypertrophy in both groups, but there was no significant measurable effect between the group given paraffin oil and those given the extract. This led to a study investigating the efficacy of these extractives. By increasing dosage and using different solvent extractions, the next clinical trial improved quality of life for patients, with some side effects (Gerber and Fitzpatrick 2004). Bayne and others (2000) performed a selectivity and specificity test on the lipido-sterolic extract of *Serenoa repens* on prostate epithelial and fibroblast cells and also investigated morphology changes in breast, kidney, skin, testes, and epididymis. In that study, changes occurred within 24 hours, but not enough changes occurred to conclude that lipido-sterolic chemicals from *Serenoa repens* made significant changes in prostate cells. Lipido-sterolic chemicals were from a hexane fraction (Bayne and others 2000, Chua and others 2014). Other solvent fractions bring about different side effects and improvements. Gas chromatography coupled with mass spectrometry has been used to identify complex mixtures. However, trace chemicals have not been detected (Moore and others 2015a, 2015b, 2017a, 2017b, 2017c). An instrument with more resolution will identify these chemical mixtures. In this study, two-dimensional gas chromatography (GC×GC) was used to qualitatively analyze chemical composition of saw palmetto extracts produced with two different solvent mixtures, acetone with water and hexane with isopropyl alcohol. The increase in peak capacity and resolving power from this analysis identified differences between the two extracts. GC×GC analysis was used to compare these fractions and identify their similarities and differences.

Experimental

Materials

Live saw palmetto plants in moist soil were purchased from a nursery. These plants were kept in a green house and watered regularly. When the moisture content fell below 30%, plants were considered to be dead. All solvents were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Tetracosane 99%, purchased from Sigma-Aldrich (St. Louis, Missouri, USA), was the calibration standard used for GC×GC.

Extraction Procedure

Saw palmetto leaves were extracted by acetone and water (9:1) and hexane and isopropyl alcohol (50:50) solutions. The leaves were cut and stored in a freezer. The leaves were removed from the freezer, slightly thawed, and weighed. The weight of leaves was calculated on the basis of dry mass (not shown). The leaves were placed in a 250-mL Erlenmeyer flask with 100 mL of solvent solution. Each extraction solution was filtered and filled with fresh solvent every 24 hours and shaken (orbital shaker) for a total of 48 hours. Extraction solutions were evaporated using a Rotavapor (Buchi Corporation, New Castle, Delaware, USA), transferred to preweighed vials, sealed, and stored in a freezer.

Instrumentation Operations

The instrument parameters used for the analysis of saw palmetto extracts are given in Table 1. The injection split and the modulation varied for different samples. The injection splits depended on the concentration of the samples. The modulation time was less than 5 seconds for each run. The time changed based on chemicals retained on the second-dimension column.

Table 1. 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)

Results and Discussion

The extractions of the plant leaves were carried out with one of two solvent solutions. These solvent mixtures were used to remove as much of the extracts from the leaves as possible. The GC×GC-time of flight mass spectrometer (TOFMS) was calibrated with a known concentration of tetracosane. Both extracts were injected in replicates. NIST and Wiley libraries were used to establish spectral similarity of the chemicals identified in the saw palmetto complex extract mixtures. The minimum similarity for matches was 700. There were 1,245 and 1,528 chemicals identified from the acetone (spa) and hexane (sph) extracts, respectively. Table 2 lists the number of chemicals detected at various levels of similarity matches. There were 509 and 636 unknown chemicals detected for spa and sph extracts, respectively.

Because of high concentrations and tailing, some compounds were duplicated. All the chemicals identified more than once were removed from the list of chemicals. All chemicals with isomers were not included in the final list. From the list of chemicals detected, 76 and 93 were left with similarity matches exceeding 800 in spa and sph extracts, respectively. There were other chemicals identified with a similarity less than 800 that are not listed. Ninety-nine chemicals were shared between extracts. These chemicals were not included in the total for each extract.

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 (acetone extract (spa) and hexane extract (sph))

Extract	Total >700	Peaks unknown	Peaks known	>800 known	>800 known[a]
spa	1,245	509	736	435	76
sph	1,528	636	892	564	93

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

The chemicals in Tables 3 and 4 are from the acetone and hexane solvent extractions, respectively. There are no duplicates, and the chemicals are specific to the solvent extracts. The chemicals listed have spectral matches greater than 900. The percentage peak area was calculated using the sum of all chemicals with matches greater than 800 similarity. The extracts did not yield many spectral matches with 900 similarity. In acetone extracts, 2,2-dimethyl-1,3-dioxolane-4-methanol 20.6%; isopropyl pyruvate 29.1%; and 1,3- 4-propyl-benzenediol 10.8% (not shown in Table 3) had significant peak areas. In hexane extracts, only one chemical, dihydroxyacetone 12.0%, had a significant peak area. Fructose 21.8% (not shown in Table 4) was also significant.

The number of saw palmetto shared chemicals was greater than the number of extraction solvent specific chemicals. There were 99 chemicals shared by both extraction systems. Fifty of the chemicals had a spectral match at the 900 similarity level and are listed in Tables 5 and 6. Pyro-catechol 16.5%, glycerin 6.12%, phenol 21.3%, and 1,3,5-benzenetriol 4.44% had the highest peak area in the 900 similarity matches. 4-propyl phenol 8.48% also had a significant peak area even though it was below the 900 similarity. Table 6 shows that phenol 8.83%, scyllo-inositol 5.86%, and pyro- catechol 10.1% were significant. Another chemical with high peak area was b-d-glucopyranose 10.3%, which is not shown in Table 6 because of the similarity cut-off.

Figures 1 and 2 show different ways to visually demonstrate the GC×GC chromatograms of the extracts. Figure 1 is a surface plot, and Figure 2 is a contour plot. The retention times of the chemicals eluted in the first dimension are based on volatility and polarity in the second dimension. 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.

Two column GC×GC chromatograms display different regions (areas) for various classes of chemicals found in a complex mixture based on the specific set of columns used. This is called molecular chemical mapping (MCM). This type of mapping can assist with unidentified chemicals in

Table 3. Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of compounds specifically identified from saw palmetto acetone extracts >900 similarity

Number	Chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	4-hydroxy-4-methylpentane-2-one	$C_6H_{12}O_2$	0.766	204.998 0.979	929
2	Isopropyl alcohol	C_3H_8O	2.888	219.991 0.941	916
3	1,3-Dioxolane-4-methanol, 2,2-dimethyl-	$C_6H_{12}O_3$	20.620	274.965 1.122	938
4	2-Propanol, 1-(2-methoxy-1-methylethoxy)-	$C_7H_{16}O_3$	0.117	329.938 1.075	910
5	D-Limonene	$C_{10}H_{16}$	0.265	354.927 0.956	929
6	(E)-4-(1',1'-Bis(ethoxycarbonyl) ethyl)-4-dodecen-2-one	$C_{20}H_{34}O_5$	0.491	364.922 1.397	963
7	1-(2-Furanyl)-2-hydroxyethanone	$C_6H_6O_3$	0.161	399.905 1.563	912
8	Ethane, 1,1'-oxybis-	$C_4H_{10}O$	0.034	409.901 0.612	908
9	methyl-(3R)-(-)-3-ethyl-5-oxopentanoate	$C_8H_{14}O_3$	0.026	504.856 0.584	906
10	4-Hydroxy-3-methoxybenzaldehyde	$C_8H_8O_3$	0.605	669.778 1.810	924
11	Benzyl benzoate	$C_{14}H_{12}O_2$	0.254	929.655 1.749	930
12	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	$C_{30}H_{50}$	2.030	1,464.4 1.378	939
13	(1,2-Dihydroxyethyl)oxirane	$C_4H_8O_3$	2.939	1,569.35 0.491	991

Table 4. Two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC TOFMS) list of compounds specifically identified from saw palmetto hexane extracts >900 similarity

Number	Chemical identified	Formula	Peak area (%)	Retention time (s)	Similarity
1	1-(Acetyloxy)-2-propanone	$C_5H_8O_3$	0.353	221.009 1.173	917
2	2-Furancarboxaldehyde	$C_5H_4O_2$	0.280	227.011 1.056	928
3	Dihydroxyacetone	C_3H_6O3	13.034	233.014 1.409	904
4	5-Allyltetrahydrofuran-2-one	$C_7H_{10}O_2$	0.042	236.015 1.408	916
5	Heptanal	$C_7H_{14}O$	0.331	245.019 0.952	946
6	Butyrolactone	$C_4H_6O_2$	0.134	257.024 1.679	945
7	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	0.880	263.026 1.320	915
8	2-Heptenal, (Z)-	$C_7H_{12}O$	0.125	290.037 1.073	930
9	1,2,3-Propanetriol	$C_3H_8O_3$	4.957	335.056 1.270	906
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	1.782	452.104 1.501	927
11	Butanedioic acid	$C_4H_6O_4$	0.530	461.108 1.303	908
12	(4R*,5R*,6R*)-4-acetoxy-1,7-dioxaspiro[5.5]undecan-5-ol	$C_{11}H_{18}O_5$	0.084	461.108 1.312	963
13	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	0.217	668.194 1.820	915
14	Vanillic acid	$C_8H_8O_4$	0.311	782.241 1.760	913
15	Muco-Inositol	$C_6H_{12}O_6$	2.042	857.272 1.849	922
16	Tetracosane	$C_{24}H_{50}$	0.662	1,268.44 0.974	927
17	Octadecanal	$C_{18}H_{36}O$	0.935	1,547.56 1.505	903
18	1,3-Dioxane, 4-ethenyl-2,2-dimethyl-6-phenyl-, cis-	$C_{14}H_{18}O_2$	0.034	1,562.56 2.054	936
19	Tetratetracontane	$C_{44}H_{90}$	3.858	1,565.57 1.308	949
20	1-Cyclohexene-1-carboxaldehyde, 2-(3,3-dimethyl-1-butenyl)-, (E)-	$C_{13}H_{20}O$	0.491	1,625.59 0.597	948
21	2-Ethylhexanal ethylene glycol acetal	$C_{10}H_{20}O_2$	2.248	1,643.6 0.491	931
22	Furan, 2,3-dihydro-	C_4H_6O	2.566	1,703.62 0.212	999
23	1-Docosene	$C_{22}H_{44}$	2.228	1,733.63 2.748	906

Table 5. Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of shared compounds identified from saw palmetto acetone extracts >900 similarity

Number	Chemical identified (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
1	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	7.168	200 3.576	958
2	(Furan-2-yl)methanol	$C_5H_6O_2$	0.066	214.993 1.071	930
3	2-Propanone	C_3H_6O	0.470	244.979 3.107	972
4	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	4.123	254.974 0.678	907
5	2(5H)-Furanone	$C_4H_4O_2$	0.613	259.972 1.706	961
6	Benzene, (1-methylethyl)-	C_9H_{12}	0.353	264.969 0.975	918
7	Glycerin	$C_3H_8O_3$	6.128	294.955 1.477	961
8	Phenol	C_6H_6O	21.289	314.946 1.212	964
9	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	0.267	319.943 1.812	904
10	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	0.117	324.941 1.470	913
11	4-Hydroxy-5,6-di-hydro-(2H)-pyran-2-one	$C_5H_6O_3$	0.032	324.941 1.525	915
12	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	0.698	329.938 1.210	917
13	D-Limonene	$C_{10}H_{16}$	0.075	354.927 0.956	929
14	Benzenemethanol	C_7H_8O	0.337	359.924 1.403	929
15	3,4-Dimethylmaleic Anhydride	$C_6H_6O_3$	0.108	359.924 1.592	931
16	p-Cresol	C_7H_8O	0.120	394.908 1.298	931
17	Phenol, 2-methoxy-	$C_7H_8O_2$	0.884	409.901 1.403	904
18	Undecane	$C_{11}H_{24}$	0.144	414.898 0.810	915
19	Nonanal	$C_9H_{18}O$	0.041	414.898 1.061	919
20	Anhydro - sugar	$C_5H_8O_4$	0.049	414.898 1.904	937
21	5-(Hydroxymethyl)dihydro-2(3H)-furanone	$C_5H_8O_3$	0.027	489.863 2.157	902
22	(pyro)-catechol	$C_6H_6O_2$	16.485	499.858 1.462	917
23	Benzofuran, 2,3-dihydro-	C_8H_8O	0.778	514.851 1.467	917
24	5-Hydroxymethylfurfural	$C_6H_6O_3$	0.703	524.846 1.838	912
25	Nonanoic acid	$C_9H_{18}O_2$	0.027	549.834 1.070	909

Table 5. Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of shared compounds identified from saw palmetto acetone extracts >900 similarity—con.

Number	Chemical identified (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
26	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.383	599.811 1.493	901
27	1,3-Isobenzofurandione	$C_8H_4O_3$	0.065	604.808 1.923	950
28	Benzaldehyde, 4-hydroxy-	$C_7H_6O_2$	0.257	629.797 1.773	942
29	1-(4-Hydroxyphenyl)-2-propanone	$C_9H_{10}O_2$	0.249	704.761 1.797	944
30	Benzoic acid, 3-hydroxy-	$C_7H_6O_3$	0.115	719.754 1.686	902
31	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	1.903	724.752 2.199	931
32	p-Hydroxybenzoic Acid	$C_7H_6O_3$	1.134	739.745 1.742	916
33	1,3,5-Benzenetriol	$C_6H_6O_3$	4.444	799.716 2.080	961
35	l-Inositol	$C_6H_{12}O_6$	3.186	909.664 2.152	928
36	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.018	994.624 0.944	900
37	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.698	1,039.6 1.103	930
38	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	0.851	1,124.56 1.086	947
39	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	0.660	1,139.56 1.286	923
40	Tricosane	$C_{23}H_{48}$	0.288	1,219.52 0.953	950
41	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.067	1,254.5 1.303	903
42	Pentacosane	$C_{25}H_{52}$	0.381	1,319.47 0.988	955
43	Octacosane	$C_{28}H_{58}$	0.358	1,409.43 1.048	937
44	Triacontane	$C_{30}H_{62}$	0.150	1,529.37 1.219	910
45	Heptacosane	$C_{27}H_{56}$	0.205	1,569.35 1.297	928
46	dl-α-Tocopherol	$C_{29}H_{50}O_2$	1.490	1,589.34 2.198	908
47	Hentriacontane	$C_{31}H_{64}$	0.059	1,604.34 1.411	916
48	1,2,4,5-Tetrakis(4-phenylphenyl)benzene	$C_{54}H_{38}$	0.563	1,644.32 0.436	992
49	β-Sitosterol	$C_{29}H_{50}O$	0.456	1,674.3 3.095	924

Table 6. Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of shared compounds identified from saw palmetto hexane extracts >900 similarity

Number	Chemical identified (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
1	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	11.940	203.001 0.732	947
2	2-Furanmethanol	$C_5H_6O_2$	0.128	212.005 1.072	909
3	2-Propanone	C_3H_6O	1.041	254.022 2.115	962
4	2(5H)-Furanone	$C_4H_4O_2$	0.323	257.024 1.726	945
5	Benzene, (1-methylethyl)-	C_9H_{12}	0.881	266.027 0.975	918
6	Glycerin	$C_3H_8O_3$	4.379	293.039 1.486	962
7	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.249	308.045 1.120	904
8	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	0.241	320.05 0.648	945
9	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	0.075	320.05 1.492	901
10	Ethanol, 2-(2-ethoxyethoxy)-	$C_6H_{14}O_3$	0.114	329.053 1.212	902
11	Limonene	$C_{10}H_{16}$	0.140	353.063 0.958	928
12	Benzenemethanol	C_7H_8O	0.125	356.065 1.422	912
13	3,4-Dimethylmaleic anhydride	$C_6H_6O_3$	0.062	362.067 1.576	929
14	Phenol, 2-methoxy-	$C_7H_8O_2$	1.190	407.086 1.416	904
15	Undecane	$C_{11}H_{24}$	0.271	410.087 0.817	941
16	Anhydro - sugar	$C_5H_8O_4$	0.177	410.087 1.940	906
17	Nonanal	$C_9H_{18}O$	0.245	416.089 1.058	955
18	(Pyro)-catechol	$C_6H_6O_2$	10.063	494.122 1.491	918
19	Benzofuran, 2,3-dihydro-	C_8H_8O	0.735	512.129 1.480	922
20	5-Hydroxymethylfurfural	$C_6H_6O_3$	0.717	521.133 1.865	923
21	Nonanoic acid	$C_9H_{18}O_2$	0.108	551.145 1.066	939
22	1,2-Benzenediol, 4-methyl-	$C_7H_8O_2$	0.846	572.154 1.514	927
23	1,3-Isobenzofurandione	$C_8H_4O_3$	0.076	602.167 1.939	924
24	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	0.301	626.176 1.717	917
25	Benzaldehyde, 4-hydroxy-	$C_7H_6O_2$	0.211	629.178 1.774	927

Table 6. Two-dimensional gas chromatography-time of flight mass spectroscopy (GC×GC TOFMS) list of shared compounds identified from saw palmetto hexane extracts >900 similarity—con.

Number	Chemical identified (shared)	Formula	Peak area (%)	Retention time (s)	Similarity
26	1-(4-Hydroxyphenyl)-2-propanone	$C_9H_{10}O_2$	0.212	701.208 1.820	945
27	3-Ethoxy-4-hydroxy-benzaldehyde	$C_9H_{10}O_3$	0.030	713.212 1.781	900
28	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	1.750	722.216 2.215	929
29	p-Hydroxybenzoic acid	$C_7H_6O_3$	1.458	737.222 1.754	915
30	Phenol	C_6H_6O	8.831	845.267 0.588	911
31	l-Inositol	$C_6H_{12}O_6$	2.785	866.276 1.800	916
33	Shikimic acid	$C_7H_{10}O_5$	1.319	917.297 2.155	908
34	Scyllo-inositol	$C_6H_{12}O_6$	5.865	929.302 1.997	912
35	2-Pentadecanone, 6,10,14-	$C_{18}H_{36}O$	0.429	968.318 1.035	915
37	Hexadecanoic acid	$C_{16}H_{32}O_2$	1.030	1,037.35 1.108	928
38	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	1.252	1,124.38 1.088	948
39	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	0.245	1,133.39 1.240	926
40	9,12,15-Octadecatrienoic acid,	$C_{18}H_{30}O_2$	1.013	1,139.39 1.288	923
41	Octadecanoic acid	$C_{18}H_{36}O_2$	0.195	1,148.39 1.136	913
42	Phenyl β-d-alloside	$C_{12}H_{16}O_6$	0.205	1,154.4 2.456	910
43	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	0.168	1,253.44 1.306	922
44	Pentacosane	$C_{25}H_{52}$	0.410	1,361.48 1.025	939
45	Octacosane	$C_{28}H_{58}$	0.547	1,406.5 1.055	939
46	Triacontane	$C_{30}H_{62}$	0.992	1,529.55 1.219	926
47	dl-α-Tocopherol	$C_{29}H_{50}O_2$	0.482	1,589.58 2.199	907
48	Hentriacontane	$C_{31}H_{64}$	1.044	1,604.58 1.412	945
49	Stigmasterol	$C_{29}H_{48}O$	0.160	1,646.6 2.845	901
50	β-Sitosterol	$C_{29}H_{50}O$	0.629	1,673.61 0.104	918

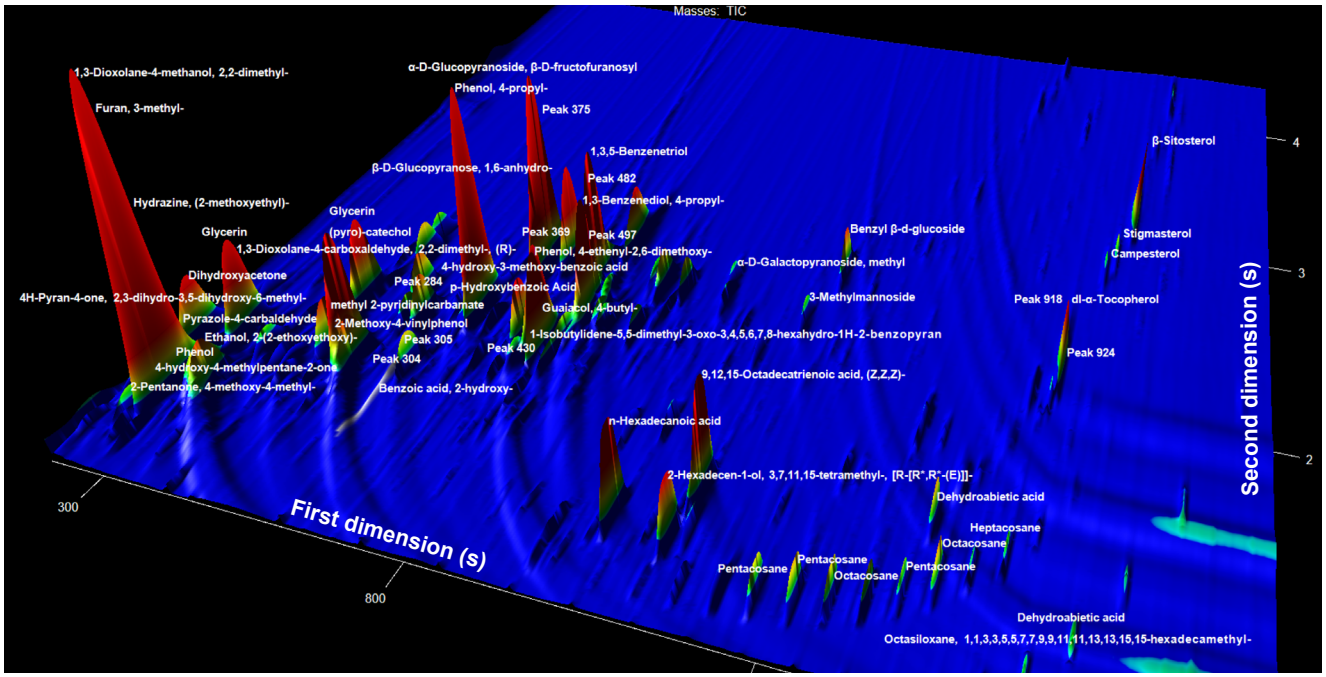


Figure 1. Two-dimensional gas chromatography (GC×GC) chromatogram surface plot for saw palmetto acetone extracts.

those specific areas. GC×GC chromatograms can be used to create a fingerprint for various complex mixtures extracted from various biomasses. This is one of the best ways to visually and chemically identify unknown chemicals in complex mixtures.

Saw palmetto has a standard GC×GC profile of extracts. In Figure 1, on the bottom right, are alkenes eluting according to the volatility. On the top right are the sterols. In the bottom middle are the fatty acids. Between 300 and 800 seconds, the area is more compact with various classes of chemicals. The majority of the chemicals between 2 and 3 seconds in the second dimension are aromatic chemicals with alcohols, acids, and methoxy groups as the ring substituents.

Figure 2a is a zoom-in capture of chemicals between 200 and 600 seconds in the first dimension. There are structures of chemicals that have been identified. These known chemicals can assist with determining the structure of the unidentified chemicals. Figure 2b first dimension retention range is 700 to 1,200 seconds. In the top left corner between 1.8 and 2.8 seconds in the second dimension, there are several identified pyranose and sugar chemicals. In the bottom right are long alkane and aldehyde chains.

Identifying chromatogram regions for classes of chemicals (RCC) located on the GC×GC chromatograms is important when characterizing a complex mixture. GC×GC is being used to create a standard way to establish a fingerprint for complex mixtures. Because of the resolving power and the visualization provided by MCM of a GC×GC chromatogram, we can better identify chemicals in extracts. This development will change the way we investigate complex mixtures.

Conclusions

Saw palmetto is known for producing chemicals that can relieve urological disorders. We were able to extract and identify the fatty acids and phytosterol chemicals in saw palmetto leaves. The concentration of extractives in saw palmetto was low, but GC×GC TOFMS was able to detect and receive spectral matches of the chemicals of interest. There were 1,245 and 1,528 chemicals detected in the spa and sph extracts, respectively. There were 509 and 636 unknown chemicals detected. GC×GC expanded peak capacity was capable of detecting and identifying trace chemicals in the extracts. The two orthogonal columns increased the peak capacity, allowing trace components to be observed and detected in the complex and low-concentration chemical mixtures. One of the best techniques for characterizing saw palmetto extracts is GC×GC TOFMS. Thousands of chemicals were identified through this technique. Future work will include determining the chemical names for the unknown chemicals identified in this study, increasing the concentration, and testing the extracts for other biological uses.

Acknowledgments

The University of Wisconsin-Madison and the USDA Forest Service, Forest Products Laboratory provided research support. Saw palmetto leaves were supplied by Mark Dietenberger. Fred Matt provided full chemical analyses of saw palmetto results for comparison. The authors appreciate Zinan Yu for GC×GC sorting and data collection. This research was presented at the International Symposium on Wood, Fibre and Pulping Chemistry (ISWFPC) in Tokyo, Japan, and was submitted for the proceedings.

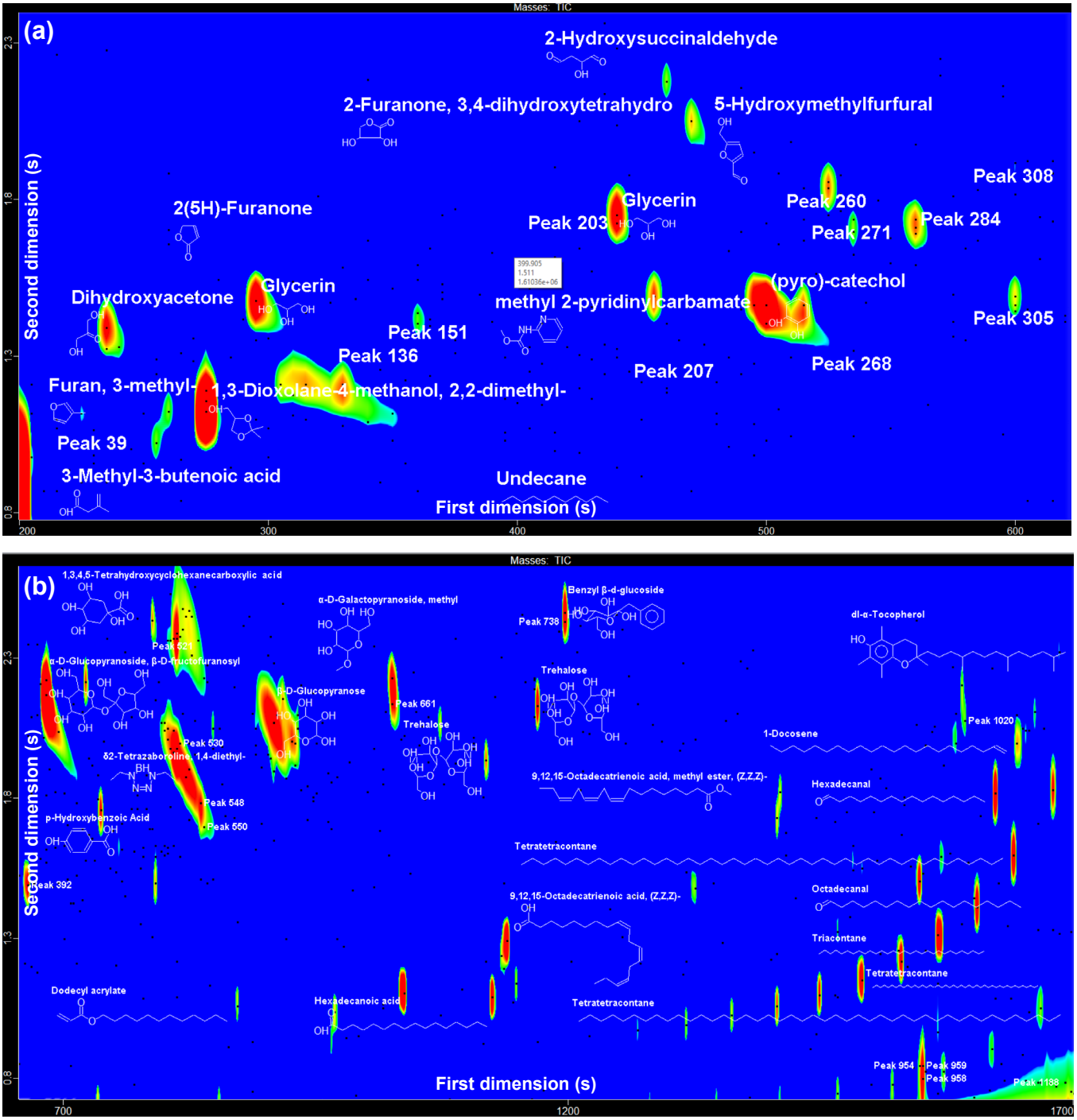


Figure 2. Two-dimensional gas chromatography (GC×GC) chromatogram contour plots for saw palmetto hexane extracts (a) zoomed in 200–600 seconds and (b) zoomed in 700–1,200 seconds.

References

Bayne, C.W.; Ross, M.; Donnelly, F.; Habib, F.K. 2000. The selectivity and specificity of the actions of the lipido-sterolic extract of Serenoa repens (Permixon) on the prostate. The Journal of Urology. 164: 876-881.

Chua, T.; Eise, N.T.; Simpson, J.S.; Venture, S. 2014. Pharmacological characterization and chemical fractionation of a liposterolic extract of saw palmetto (Serenoa repens): Effects on rat prostate contractility. Journal of Ethnopharmacology. 152: 283-291.

Gerber, G.; Fitzpatrick, J. 2004. The role of a lipido-sterolic extract of Serenoa repens in the management of lower urinary tract symptoms associated with benign prostatic hyperplasia. BJU International. 94: 338-344.

Moore, R.; Leitch, M.; Arellano-ruiz, E.; Smaglick, J.; Mann, D. 2015a. Mountain pine beetle infestation: GC×GC-TOFMS and GC-MS of lodgepole pine (pinus contorta) acetone extractives. In: 18th international symposium on wood, fiber and pulping chemistry. September 7-11. Vienna, Austria. 254-259.

Moore, R.; Smaglick, J.; Arellano-ruiz, E.; Leitch, M.; Mann, D. 2015b. Chemical polarity vs wood durability: The polarity of extractives on the durability of wood. In: 18th international symposium on wood, fiber and pulping chemistry. September 7-11. Vienna, Austria. 375-378.

Moore, R.; Mann, D.; Epstein, G.; Hinkforth, B.; Wagner, P.; Hyunji, J. 2017a. Characterization of extractives in durable and non-durable hardwoods: black locust, catalpa, and honey mesquite. In: 19th international symposium on wood, fiber and pulping chemistry. Aug. 28-Sept 01, 2017. Porto Seguro, Bahia-Brazil. 5 p.

Moore, R.; Mann, D.; Epstein, G.; Hinkforth, B.; Wagner, P.; Hyunji, J. 2017b. Comparative characterization of extractives in Alaskan yellow, eastern red, and western red cedars. In: 19th international symposium on wood, fiber and pulping chemistry. Aug. 28-Sept 01, 2017. Porto Seguro, Bahia- Brazil. 4 p.

Moore, R.; Mann, D.; Yilgor, N. 2017c. Comparative study of fungal deterioration in liquidambar orientalis mill heartwood extractives. In: 19th international symposium on wood, fiber and pulping chemistry. Aug. 28-Sept 01, 2017. Porto Seguro, Bahia-Brazil. 3 p.

Willetts, K.E.; Clements, M.S.; Champion, S.; Ehsman, S.; Eden, J.A. 2003. Sernoa repens extract for benign prostate hyperplasia: a randomized controlled trial. BJU International. 92: 267-270.