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ANALYSIS OF SOUTHWESTERN COLORADO *LIGUSTICUM PORTERI* BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Abstract

Osha root (*Ligusticum porteri*) is a perennial herb found in parts of the Rocky Mountains. It is used as an immune booster and a treatment for colds, flus, indigestion, and body aches. In this study we determined compounds present in osha root from the San Juan Mountains in Southwestern Colorado by Gas Chromatography – Mass Spectrometry. Extractions were performed using hexane and methanol solvents at room temperature. Some thirty to forty compounds were detected. Eleven compounds with a high search indices are discussed and compared with previous studies.

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Introduction

The uses of *Ligusticum porteri* have been known for many years by the Native American tribes in the Rocky Mountain areas of the US and Mexico. The general name for this plant came from observing bears exposing the root and rubbing their fur on it to expel any parasites they may have acquired during hibernation.[1] Hence, the name osha root being that "osha" translates to bear in some Native American languages.[2]

Medicinal folklore of this plant tells of treatment of pain, upper respiratory disease, gastrointestinal issues, and pancreas disease.[3] The qualitative analysis completed by Gas Chromatography – Mass Spectrometry (GC-MS) from polar and non-polar solvent extracts show that there are many useful compounds found in this plant.

Cordero[1] analyzed the chemical components of the osha plant and compared the results to the related species *L. grayi*. The harvested samples were taken from the northern and central region of California including the Sierra Nevada Mountain range. Simple extractions (by letting the dried root soak in solvent) and Soxhlet extractions were used. The solvents were hexane, dichloromethane, and ethanol. The extracts were analyzed by Gas Chromatography – Mass Spectrometry (GC-MS), and Gas Chromatography – Flame Ionization Detection (GC-FID).

Collin et al.[4] identified compounds of *L. porteri* through solvent extraction and GC-MS / GC-FID analysis. Anhydrous ethanol and acetone were utilized as the solvent agents. The sample lot was purchased from a seller in Eugene, Oregon. They also produced hydrosol through steam distillation which was used for comparison purposes against the solvent extracts.

Delgado et al.[5] conducted GC-MS analysis of hexane solvent extractions. Their sample material was sourced from Sierra Tarahumara, Mexico.

In our work reported in this paper, methanol and hexane solvent extractions at room temperature were used. Since most people that use the osha root will chew it or make a tea with it, we decided to use methanol extraction solvent. Methanol is structurally similar to water yet compatible with use in GC-MS. To compare with previous studies and any possible effects on solvent polarity hexane was also used. Room temp extraction was chosen so that volatile compounds might be retained. Our osha root was sourced from the San Juan Mountains in Southwest Colorado which has not been previously studied. The exact location is being withheld due to over harvesting concerns.

Experimental Methods

For the solvent extractions, 1.0 gram of dried root and rhizomes were finely chopped and added to 10.0 mL hexane or methanol in a sealed sample jar. The sample was left to soak for 24 hours at 23°C. Using a syringe, the liquid was withdrawn and filtered through a 0.45 um syringe filter into a sealed vial for injection into the GC.

GC-MS analysis was conducted using a Thermo Trace GC Ultra Polaris Q equipped with a Restek Rxi-5Sil, 30-meter, 0.25 mm internal dimension column. The carrier gas was helium with a flow rate of 1 mL per minute. The injector temperature was set at 300°C with a 10:1 split ratio. The sample injection volume was 1.00 μL. The temperature program was: initial oven temperature at 40°C, hold for one minute, temperature ramp at 20°C per minute to 300°C, hold for one minute. The transfer line temperature was set at 300°C. The ion source temperature was 200°C. The mass range selection was 20-200 m/z. The results were analyzed by Thermo Excalibur software using total ion count ICIS peak detection / integration with 15 smoothing points, baseline window of 40, area noise factor of 5, and peak noise factor of 10. Mass spectra were searched using a National Institute of Standards and Technology (NIST) 2017 library. Blank injections of solvent only were also undertaken. Peaks present in the blank were eliminated from identification.

Results and Discussion

Figure 1 and Figure 2 show the chromatograms of hexane and methanol extracts respectively typical of replicate runs. The numbered peaks are what we have a high confidence of identification based on the top hit list rankings generated by the Thermo Excalibur software matching to the NIST 2017 library.

Three factors describe the accuracy of the Excalibur match:
SI, A direct matching factor for the unknown and the library spectrum.
RSI, A reverse search matching factor ignoring any peaks in the unknown that are not in the library spectrum.
Prob, A probability factor based on the differences between adjacent hits in an SI ordered list.

With the SI and RSI matching factors, a perfect match results in a value of 1000. As a general guide 900 or greater is an excellent match; 800–900, a good match; and 700–800, a fair match. A matching factor less than 600 is a poor match. Unknown spectra with many peaks tend to yield lower match factors than otherwise similar spectra with fewer peaks. The probability factor is a complex parameter based on the SI matching factor and the difference between adjacent matches. If a hit has an SI match factor > 900 and the next best hit has a match factor of 300, the probability of the compound being correctly identified is high. Conversely, if several hits are returned with very similar SI matching factors, the probability of a correct assignment is low. In our data we chose to report the top probability matches with RSI over 830.

The GC-MS analysis of the hexane and methanol extracts identified eleven compounds with an RSI value of 832 or greater. Compounds numbered 1 to 9 are present in both samples. Compounds 10 and 11 are solely in the methanol extract. Figure 3 lists the compounds by peak number providing the name, the relative search index, which extract they are found in, and the peak area percentage of the sample. Figure 4 shows the compound structures correlated by peak number. Most of the compounds are terpenes with the exception of compounds 7, 10, and 11 which are methoxy substituted benzenes with 10 and 11 being substituted phenols. The compounds from the two largest peaks in both chromatograms (surrounding peak 9 at retention times 10.4 and 10.8 respectively) may be terpenes however, the SI and RSI factors were near or below 800 and therefore not confidently identified in this work.

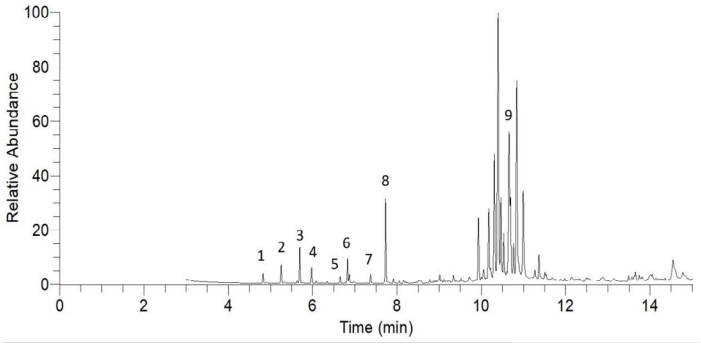

Figure 1. Chromatogram of hexane solvent extract of osha root.

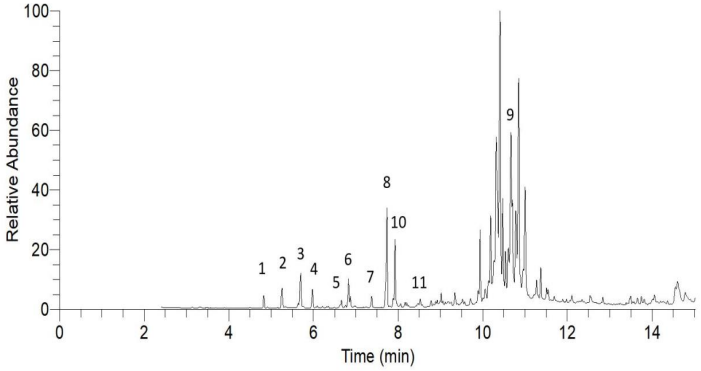

Figure 2. Chromatogram of methanol solvent extract of osha root.

Of the compounds identified most have a known medical, industrial, food, or cosmetic use. Compound 1 (2-Thujene) has a registered patent for its use in treating pain related to musculoskeletal disease.[6] Compound 3 (p-Cymene) can be used as a flavoring agent, but the primary use is a fragrance in home air fresheners and surface cleaners.[7] Compound 4 (3-Carene) is also used in fragrances and cleaning products. It is also used as a disinfectant of hard surfaces or laundry.[8] Compound 5 (4-methylene-1 bicyclo[3.1.0] hexan-3-ol) is listed in a patent involving inhibitors of human immunodeficiency virus replication.[9] Compound 9 (E-Ligustilide) has shown positive results in limiting oxidative stress in the aiding in repair of kidney tissue.[3]

		Hexane		Methanol	
Peak Number	Compound Name	RSI	Peak Area %	RSI	Peak Area %
1	4-methyl-1-(1-methylethyl)-Bicyclo[3.1.0]hex-2-ene 2-Thujene	878	0.51	878	0.53
2	4-methylene-1-(1-methylethyl)-1-Isopropyl-4-methylenebicyclo[3.1.0]hexane Bicyclo[3.1.0]hexane Sabinene	878	1.13	874	0.96
3	1-methyl-4-(1-methylethyl)-Benzene P-Cymene	908	2.28	924	2.07
4	3,7,7-trimethyl- Bicyclo[4.1.0]hept-3-ene 3-Carene	878	0.51	903	0.87
5	4-methylene-1 Bicyclo[3.1.0]hexan-3-ol	870	0.48	866	0.37
6	2-Methyl-7-exo-vinylbicyclo[4.2.0]Oct-1(2)-ene	832	1.98	846	1.49
7	2-methoxy-1-methyl-4-(1-methylethyl)-Benzene	883	0.53	904	0.48
8	(1a,3a,5a)-4methylene-1-(1-methylethyl)- Bicyclo[3.1.0]hexan-3-ol	898	5.00	878	4.45
9	E- Ligustilide	886	13.07	895	13.8
10	2-Methoxy-4-vinylphenol 4-ethenyl-2-methoxy-Phenol	x	x	916	3.33
11	3-hydroxy-4-methoxy-Benzaldehyde 3-hydroxy-p-Anisaldehyde	x	x	901	0.79

Figure 3. Table of identified compounds in hexane and methanol extracts of osha root.

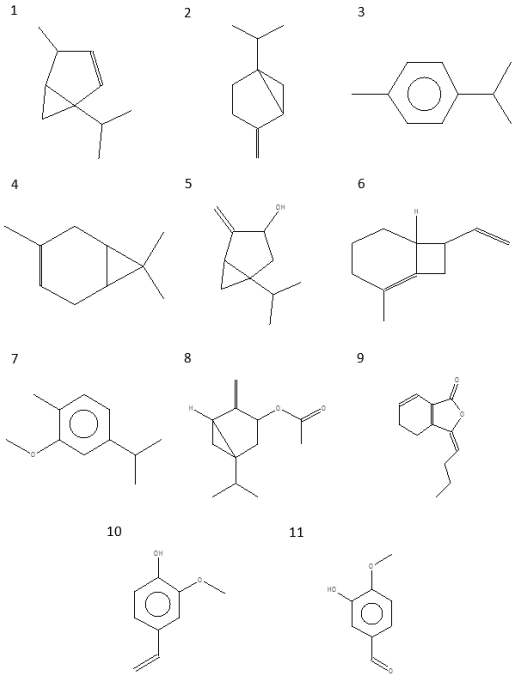

Figure 4. Compound structures of hexane and methanol extracts of osha root identified listed by peak number.

Conclusion

In comparison to Cordero's and Collin's previous studies, 1-Thujene (an isomer of 2-Thujene), p-Cymene, Sabinene, and E-ligustilide were identified and in agreement with our work reported here. Delgado identified E-ligustilide as the compound present in the largest amount in osha root extracts. E-ligustilide was also found in significant amount in our results. In contrast, compounds 4, 5, 6, 7, 8, 10, and 11 were not identified in previous papers. This could suggest that these compounds might be unique to the plants in the San Juan Mountains in Southwestern Colorado, or these compounds are more volatile and recovered in the cold extraction process.

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