The Characterization of the Chemical Constituents of Osha "Bear" Root and the Correlation to Purported Medicinal Activities

Allison Rogowski[1], James M. Chapman[1], and Kelly Kindscher[2]
1. Rockhurst University, Kansas City, MO
2. University of Kansas, Lawrence, KS

ABSTRACT
Osha root has commonly been used by Southwestern Native American and Hispanic tribes as a cure from a wide range of afflictions, from the common cold to wound infections. In an attempt to support the many medicinal claims, an investigation to ascertain the identity of other bioactive components is being carried out utilizing SPME fiber analysis of the root's volatiles when heated and dichloromethane/methanol organic extraction of the root by gas chromatography-mass spectrometry (GC-MS). Both methods have detected over 100 compounds and a number of these can be correlated to the purported medicinal uses of the plant by American Indians.

INTRODUCTION
Osha is a perennial herb found in the Southwest and Rocky Mountains, growing in deep, moist soils. Osha is also known by its scientific name *Ligusticum porteri* and by its common names, which include osha root, Porter's ligusticum, or bear root. Osha exhibits a variety of medicinal properties and has been applied as an antiviral agent, diuretic, decongestant, stimulant, and diaphoretic, among others. The root has most commonly been used by Hispanic cultures and Native American tribes such as the Apache, Zuni, and Tewa. Among the volatiles identified were etilefrine, alpha-pinene, and ligustilide, all of which have been associated with medicinal activities. Since the 1994 Dietary Health and Education Act was passed, allowing any natural substance to be deemed a "dietary supplement", research on the medicinal constituents of herbal remedies has increased dramatically. Gas chromatography-mass spectrometry (GC-MS) is commonly utilized for the identification of the volatile fractions of plant species. The methods of isolating the volatile fractions prior to introduction into the GC-MS are varied and often involve solvent extraction or distillation. Among the most common solvent extraction methods is dichloromethane/methanol organic extraction, which was utilized in this investigation. Solid phase microextraction (SPME) has proved to be a valuable tool for headspace sampling and was chosen as a second method utilized during this investigation.

INSTRUMENTATION – GCMS
The GC utilized for the analysis was a Agilent 5890 equipped with a Zebron ZB-1 column (15m x 0.25mm x 0.25um) (Phenomenex Torrance, CA). The mass spectrometer used was an unmodified benchtop quadrupole Agilent 5971 Mass Selective Detector (Agilent Technologies, Inc., Palo Alto California). The 5971 was controlled by ATX-EIGHTY-X data system (CSS Analytical Co. Inc.) with Agilent G1701BA Chemstation running on Microsoft Windows XP. The conditions for the GC were initial oven temperature of 40°C, injector 250°C, transfer line 280°C, a solvent delay of 2.00 min, the temperature was ramped at 10°C/min to a final temperature of 230°C and held for 1.00 min. The data collected from the GC-MS was analyzed with AMDIS (Automated Mass Spectral Deconvolution and Identification System), version 2.1, DTRA/NIST, 2002.

SPME SAMPLING METHODS
Solid samples of the osha root were weighed into 13x100 mm test tubes and the test tube was fitted with a rubber septum. Samples were preheated at 65°C for 15 min. Extraction was performed for 40 min in the oven by inserting a DVB/Carboxen/PDMS fiber (Supelco,Sigma-Aldrich) to absorb the volatiles in the sample headspace. The fiber was desorbed in the GC injection port for 5 min.

CONCLUSIONS
Both the SPME sampling method and GCMS analysis of the root components proved to be an effective method by which to characterize the medicinal compounds of osha root. The results of the organic extraction and volatiles present in the root that have been associated with medicinal purposes are shown in Table 1. Previous research has reported only a limited number of chemical constituents responsible for the root's medicinal uses. The percentage of compounds responsible for osha root's reported medicinal activites, over 13% of the total chemical composition of the root, could be responsible for the use of osha root for a variety of illnesses and symptoms.

RESULTS
Table 1: Compounds identified through organic extraction and volatiles analysis that have been correlated with medicinal properties.

Compound	Common Name	Medicinal Uses
1(3H)-Isobenzofuranone, 3-butylidene-	Ligustilide	tonic for colds, flu, bronchial infections, fever, poor digestion, aches and pains, cough, bronchial conditions, fevers painful menstruation, retained placenta, fevers,digestive disorders, toothache
1,2,5-Oxadiazole	Furazan	Derivatives show the following properties: antimalarial, antihistamine, antiinflammatory, gastrosparing, antiplatelet
1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	3,5-Di-tert-butylcatechol (DTCAT)	antispasmodic
1,2-Benzisothiazole		antibiotic, antimicrobial
1,3,6,10-Dodecatetriene, 3,7,11-trimethyl-	Nerolidol	antiulcer (for gastric ulcer treatment)
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-	Germacrene	antimicrobial
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-	(E)-Germacrene D	antimicrobial
1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-,cis-	Widdrol	antifungal
1H-Cycloprop[3]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-	Viridiflorol	anticancer (in lung carcinomas)
1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-	(-)-Dehydroaromadendrane	antifungal
2(1H)-Naphthalenone, 7-ethynyl-4a,5,6,7,8,8a-hexahydro-1,4a-dimethyl-	Chamaecynone	antifungal
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-tetramethyl-	Alpha-Eudesmol	Inhibits exocytotic glutamate, attenuates post-ischemic brain injury
2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	Cineole; Eucalyptol	Cough suppressant, reduces inflammation, pain relief, has been shown to kill leukaemic cells in vitro
4-Ethoxyphenethylamine	Ethoxyphenylamine; 4-Phenetidene	precursor to Phenacetin, an analgesic with additional properties that include a depressing effect on heart rate, antipyretic, and a treatment for rheumatoid arthritis, intercostal neuralgia, and some forms of ataxia
7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)	7-Oxabicyclo[2.2.1]heptane	antiinflammatory
7-Oxabicyclo[2.2.1]hept-2-ene, 5-methylene-	7-Oxabicyclo[2.2.1]heptane	antiinflammatory
Acetophenone, 4-hydroxy-	4-AH	potent stimulatory effects on hepatic bile secretion
Propylhexedrine	Benzedrex	vasoconstricter used for symptamatic relief of nasal congestion, anoretic properties
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	Borneol	Used in traditional Chinese medicine to promote smoother blood circulation
Butanamide, N-(4-hydroxyphenyl)-	N-Butyryl-p-aminophenol	liver regeneration
Caryophyllene	b-Caryophyllene	antiinflammatory
Cyclotetrasiloxane, octamethyl-	D4	antiperspirant, blood defoaming agent, and used as a protective sealant and lubricant for wound dressing
Diaminopyradine		proposed treatment for Lambert-Eaton syndrome, in which it functions to block potassium channel efflux in nerve terminals in order to increase action potential duration
Etilefrine		cardiac stimulant used as an antihypotensive
Eudesma-4(14),11-diene	beta-Selinene	antimalarial, antiplasmodial
Nitrous Oxide		anesthetic, analgesic
Phenol, 2-methoxy-4-(1-propenyl)-, acetate	Isoeugenol	antiseptic, analgesic
Propanoic acid	Propionic acid	antifungal
Salinazid	Salinazide	diuretic intended to lower high blood pressure

Figure 1 **SESQUITERPINES**

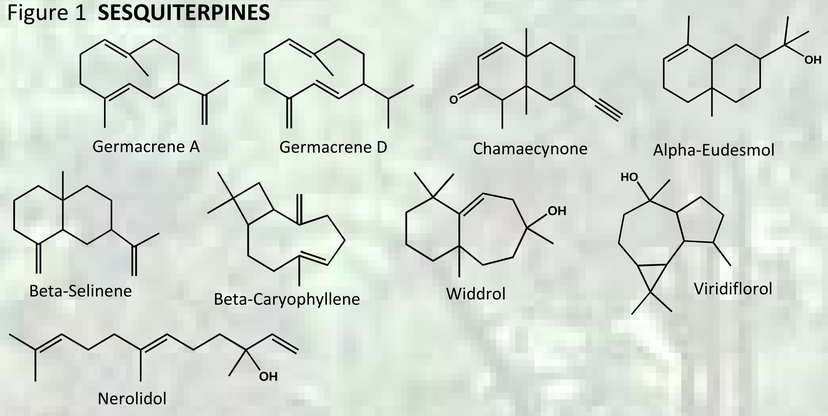


Figure 2 **PHENYLPROPANOIDS**

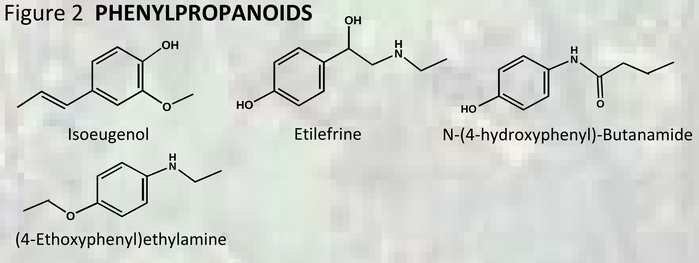


Figure 3 **HETEROCYCLES**

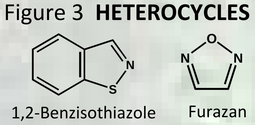


Figure 4 **MONOTERPINOIDS**

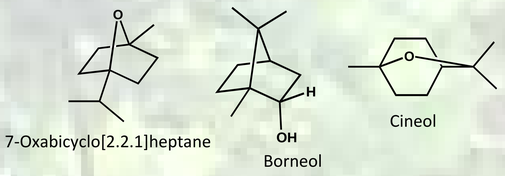
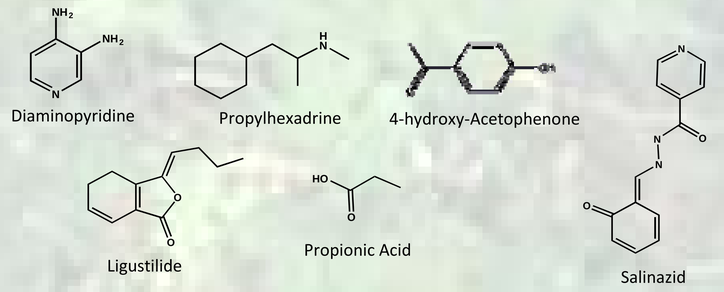


Figure 5 **MISCELLANEOUS**



Figures 1-5: Medicinal compounds isolated by GC-MS and SPME fiber analysis sorted by structural category.

FUTURE RESEARCH
We are planning to further investigate the chemical constituents of osha root by additional methods of analysis. Future research will be conducted with the goal of identifying more medicinal compounds in order to better categorize and compare their structural components.