Abstract

OilExTech contracted the senior design group to characterize and optimize essential oil extraction parameters for lodge pole pine (*Pinus contorta*), giant sequoia (*Sequoiadendron giganteum*), scotch pine (*Pinus sylvestris*), grand fir (*Abies grandis*), and western juniper (*Juniperus occidentalis*). The EssenEx-100 in-home microwave extraction unit was used to analyze plant materials for its extraction capability; juniper and grand fir yielded appreciable volumes of oil and were subject for optimization. Juniper (berries and needles) and grand fir samples were evaluated by three operators in triplicate with a design matrix using 100 and 200 g ground samples extracted for five and seven minutes. Juniper berries and needles were also analyzed separately to assess specific yield by extracting 100 g samples for five minutes. Lodge pole pine, giant sequoia, and scotch pine did not yield appreciable amounts of oil. Juniper yielded 0.20-0.40 g oil/100 g wet mass and grand fir yielded 0.05-0.30 g oil/100 g wet mass with 90% confidence. ANOVA analysis showed high variation and little correlation between oil yield, mass and time with p-values above 0.05. Juniper berries produced 0.6-1.6 g oil/100 g wet mass and juniper needles produced 0-0.4 g oil/100 g wet mass with 90% confidence. Gas chromatography was used in series with a mass spectrometer to analyze the chemical composition of the essential oil samples; four to nine primary chemicals were identified for each sample.

Background

Essential oils are hydrophobic and consist primarily of fragrant hydrocarbon and oxygenated compounds that provide natural anti-parasitic, anti-microbial, and antiviral properties for selected plants. Juniper essential oils are used for aromatherapy, treating digestive problems, disinfectant, and flavoring in gin. Grand fir essential oils are used as disinfectants, cough suppressants, they soothe muscle and joint pain, and are used in aromatherapy. Terpenoids contribute the majority of bulk aromatics, while more sensitive oxygenated compounds such as esters, aldehydes, and phenols add subtle aromas.[1] The latter compounds provide higher quality oil but are thermally sensitive. Compounds found in juniper and grand fir can be found in Appendix A4[2,3,4].

OilExTech designed the EssenEx-100, a microwave-powered essential oil home extraction unit that allows consumers to produce small quantities of essential oil in less than eight minutes from tested materials including mint, lavender, thyme, and rosemary. Pines, grand fir and juniper were tested in this study to assess extraction feasibility and expand the accredited OilExTech extraction portfolio. Essential oils are present in all parts of the plant material including the heartwood, branches, twigs, buds, berries, and needles. Highest oil concentrations are found in the needles and, with juniper, in the berries.[5]

The EssenEx-100 uses solvent free microwave extraction (SFME) to extract essential oils. Oils are volatilized and carried by steam, derived from in situ water, to a condenser in SFME. Microwaves transfer energy into the plant material via ionic conduction and dipole rotation; the former generates heat from friction created by microwave induced electron and molecular motion while the latter relies on

[1] Elliott, Ed. "Solvent Free Microwave Extraction of Essential Oils from Plant Matrices." Capstone Project. Northeastern Illinois University. Illinois. Speech.

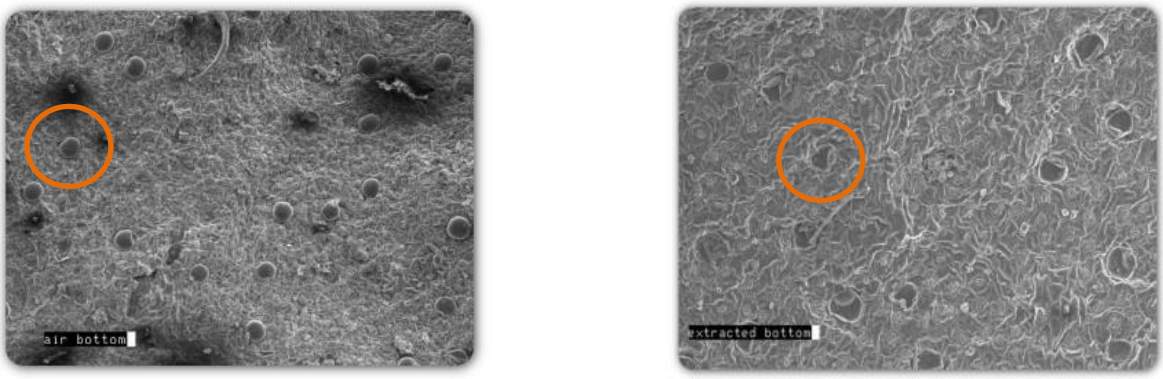
[2] Chatzopoulou, Pashalina S. and Katsiotis, Stavros T. (1995) Procedures influencing the yield and the quality of the essential oil from *juniperus communis* L. berries. Pharmaceutics Acta Helvetiae. 70, 247-253.

[3] Tatro, V.E., Scora, R.W., Vasek, F.C., and Kumamoto, J. (1973) Variations in the leaf oils of three species of juniperus. American Journal of Botany. 60, 236-241.

[4] Muzika, R.M., Campbell, C.L., Hanover, J.W., and Smith, A.L. (1990) Comparison of techniques for extracting volatile compounds from conifer needles. Journal of Chemical Ecology. 16, 2713-2722.

[5]Kelkar, Vasant, et. al. "How to recover more value from small pine trees: Essential oils and resins." *Elsevier Biomass and Bioenergy*. (2006): 316-320. Web. 17 Mar. 2013.

microwaves to alter the dipole location and 'vibrate' the molecule $4 \cdot 10^9$ times per second to generate heat. Oil-containing sacks are energized by microwaves, rupture, and release oils into the system where oils volatilize and steam carries the oils. The steam and oil vapor mixture is condensed into a liquid where the water and oil can separate into binary phases. Figure 1 shows the effect of microwaves on oil sacks in a mint leaf; the nodules are intact prior to extraction and burst after exposure to microwaves.[6]



A **B**

Figure 1: (A) shows undisturbed oil sacks, prior to microwave agitation. **(B)** Shows the broken oil sacks after sufficient microwave energy displaced the cell walls releasing the essential oil.

Microwave energy partially dissipates prior to reaching the plant material according to the dissipation factor, shown in Equation 1. The dissipation factor is represented by δ, ε'' is dielectric loss, and ε' is dielectric constant.

$$\tan(\delta) = \frac{\varepsilon''}{\varepsilon'} \qquad (1)$$

Dielectric loss describes the energy conversion efficiency of a solvent while dielectric constant describes how easily a molecule is polarized. Water, with a dissipation value of 72, inhibits energy transfer. Solvents such as hexane, with a dissipation value of 1.5, are pervious to most microwaves. Contrary to steam distillation, microwave extracted oils are exposed to lower temperatures for shorter periods of time allowing more thermally sensitive oxygenated compounds to be extracted. SFME also requires less energy because it does not involve the steam generation from an external source.1

Materials and Methods

The EssenEx-100 extraction kit was used for all extraction runs. The kit includes a 7" diameter by 7" high glass jar, a 250 mL graduated separation flask, upper and lower heat shields, two pipettes, four 0.5 dram vials, one mug, and one ice core mold. The components of the kit are pictured in Figure 2.

[6] Velasco, C. 2007. Microwave Extraction of Peppermint Oil and Comparison to the Current Practice of Steam Distillation [thesis]. Corvallis (OR): Oregon State University. 131 p.

Figure 2: Components of the EssenEx-100 Complete Extraction and Separation kit. The upper heat shield is pictured in Figure 3. The lower heat shield is pictured inside the glass jar. A frozen ice core and a 250 mL glass beaker are not pictured.

All extraction runs were performed in an 1400 watt General Electric microwave following the standard operating procedure published by OilExTech, located in Appendix A5. Plant material was weighed in a 1 L beaker on a Sartorius GE1302 digital scale then transferred into extraction unit. The lower shield was placed in the extraction unit and covered with an inverted funnel prior to adding plant material to ensure the biomass filled the annulus region of the unit and the area within the shield was vacant. The collection beaker was then placed inside the lower shield, the upper shield was placed on top of the unit, and a frozen ice core was attached to the lid. Figure 3A-B shows the extraction unit with biomass, collection beaker, ice core, and upper and lower heat shields.[7]

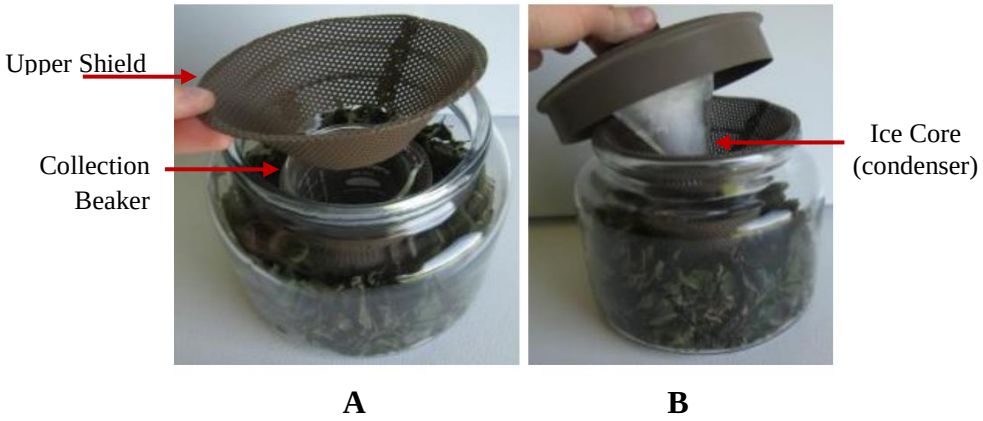


Figure 3: The EssenEx-100 extraction unit filled with mint leaves ready for oil extraction. **(A)** Shows the empty collection beaker in the center of the apparatus. **(B)** Highlights the attached ice core that is centered over the collection beaker during extraction.

The filled apparatus was placed in the microwave on high power for five or seven minutes. The mug was filled 2/3 full of water and placed in the microwave alongside the EssenEx during extraction. Protective gloves were used to remove the unit and mug from the microwave following extraction. The unit was allowed to cool at room temperature for 15 minutes. Presence of the ice core was noted after extraction and after cooling.

[7] Photo courtesy of OilExTech.

Liquid collected in the 250 mL beaker was poured into a 250 mL graduated flask where oil and water separated within two minutes; gentle flask agitation encouraged separation. Water was added to raise the fluid level into the neck of the flask and make the oil layer more visible. A pipette was used to transfer the extracted oil to 0.5 dram storage vial. The extracted oil and the post-extraction biomass were weighed to quantify oil yield and plant moisture losses, respectively. Oil yield was quantified using Equation 2.

$$Oil\ Yield = \frac{g\ oil}{100g\ wet\ plant\ mass} \qquad (2)$$

Lodge pole pine (*Pinus contorta*), giant sequoia (*Sequoiadendron giganteum*), scotch pine (*Pinus sylvestris*), grand fir (*Abies grandis*), and western juniper (*Juniperus occidentalis*) were tested for feasibility. Juniper berries and needles were tested together and separately. Needles were tested for all other species.[8] Lodgepole pine was harvested from Bend, Oregon. Giant sequoia was harvested from Corvallis, Oregon. Scotch pine and grand fir were provided by Holiday Tree Farms in Corvallis, Oregon. Juniper was harvested from Madras, Sisters, and Bend, Oregon.[9]

All samples were stored frozen in a Haier HCM071LC freezer until used. All materials were ground while frozen with a Cuisinart DLC-4CHB Mini-Prep Plus 4-Cup food processor. Juniper berries were ground further in an Oster BRLY07-B 7-Speed Fusion Blender. Grinding the bio-material disrupts the oil sacks prior to extraction and increases surface area for volatile oil diffusion.

Moisture content of all plant species was determined by drying whole samples at 150 ℃ for ~24 hours in a Blue M single wall gravity convection laboratory oven. Equation 3 was used to calculate moisture content percentages.

$$\%\ Moisture\ Content = \left(\frac{wet\ mass - dry\ mass}{wet\ mass}\right)100 \qquad (3)$$

Initial feasibility tests were performed on each species. Juniper and grand fir were then further analyzed by varying initial wet masses and extraction times. Table 2 outlines the wet masses, extraction times, number of operators, and number of replicates performed for each parameter, and total number of runs for each material.

Material	Wet Masses (g)	Extraction Times (min)	Number of Operators	Number of Replicates	Total Number of Runs
Juniper Berries With Needles	100, 200	5, 7	3	3	36
Juniper Berries	100	5	3	1	3
Juniper Needles	100	5	3	1	3
Grand Fir Needles	100, 200	5, 7	2	3	24

Table 2: Experimental design for each material analyzed. As many runs as possible were performed in the time available.

Average yields were calculated with 90% confidence intervals for each parameter tested.

A GC was used in line with JEOL MSRoute magnetic sector analyzer mass spectrometer to examine the chemical composition of essential oils in juniper berries, juniper needles, and grand fir needles. Non-diluted oil samples were directly injected into the GC column, where argon was the carrier gas, using a split ratio of 1. Column temperature was ramped from 30°C to 240 °C over a 12-minutre elution span. A

[8] Needles for all species included small twigs and branches.
[9] Juniper harvested from Bend, Oregon was provided by RR-Bar Ranch.

magnetic sector analyzer separated ions based on their momentum.[10] Compounds were separated based on their affinity for the column solid phase, longer retention times show higher affinity.

Results and Discussion

Lodge pole pine (*Pinus contorta*), giant sequoia (*Sequoiadendron giganteum*), and scotch pine (*Pinus sylvestris*) did not yield measureable volumes of oil. Grand fir (*Abies grandis*), and western juniper (*Juniperus occidentalis*) produced extractable amounts of oil. Table 3 shows the moisture content for successful materials.

Moisture Content	
Sample Type	% Moisture
Juniper (needles and berries)	48
Grand fir	56
Juniper berries	46
Juniper Needles	50

Table 3: Percent moisture content for grand fir and variations of juniper plant material. Moisture content was near 50% for all plant material tested. The high dissipation factor of water allows microwaves to transfer energy to create steam.

Figure 4A-B shows average oil yields from the experimental design for combined and separate juniper berries and needles. The number of extraction runs represented by each graph is shown in Table 2. High variations in the yield were assessed by analyzing oil yields separately for juniper needles and juniper berries. The large difference in oil yield between berries and needles seen in graph B could be responsible for the variation in oil yield for combined berry and needles extractions, shown in graph A, as the number of berries for each extraction was not consistent.

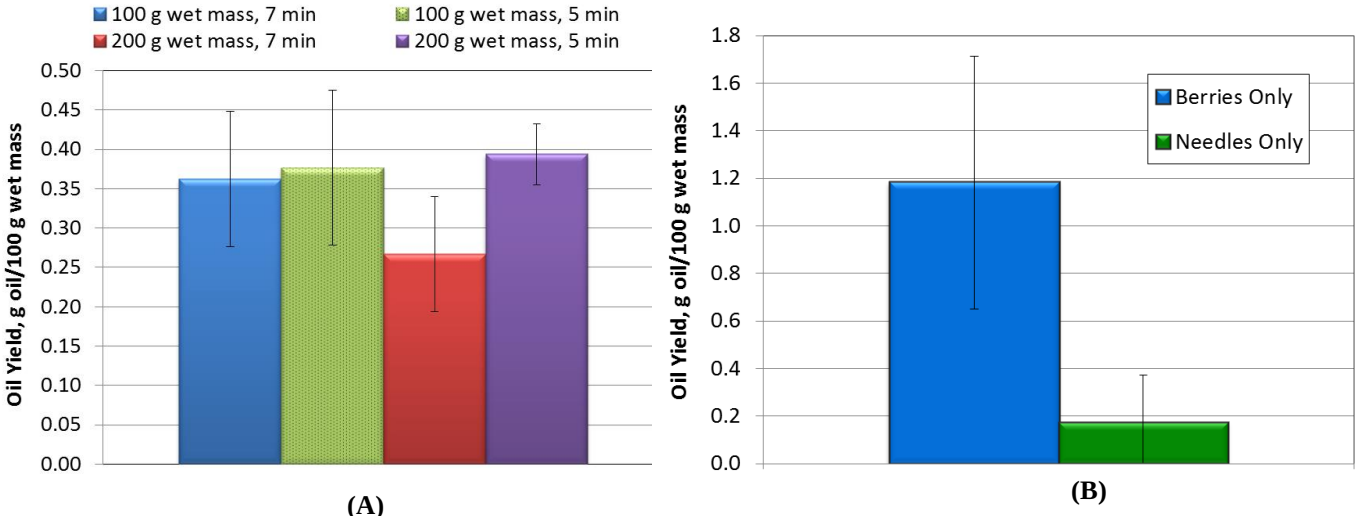


Figure 4: Average oil yield per 100 g wet mass for juniper extractions. Error bars show 90% confidence. (A) Oil yields combined juniper needle and berry extractions. Two hundred gram samples were dependent on extraction time for the where 7 min extractions yielded less oil. (B) Oil yield for separate juniper berries and needles. Berries contained five times more oil than needles.

Optimum operating parameters could not be determined for the juniper oil extraction. ANOVA analysis showed no correlation between extraction mass and time for the 100 g sample; 2-factor and one-factor analysis reported p-values greater than 0.05. ANOVA analysis showed that the 200 gram sample

[10] Jeff Morre can be contacted at jeff.morre@oregonstate.edu for specific details regarding sample analysis.

extracted for seven minutes yielded less oil than the 100 g sample; this correlation was caused by complete melting of the ice core. Juniper berries yielded 1.2 g oil/ 100 g wet mass while juniper needles yielded 0.2 g oil/ 100 g wet mass. A P-value of 0.05 showed the difference in oil yield between berries was significant. Multiple wet masses and extraction time could not be investigated for juniper berries in the allotted project time.

Figure 5 shows average oil yields from grand fir extractions. Error bars represent 90% confidence. The number of runs represented by Figure 5 is shown in Table 2.

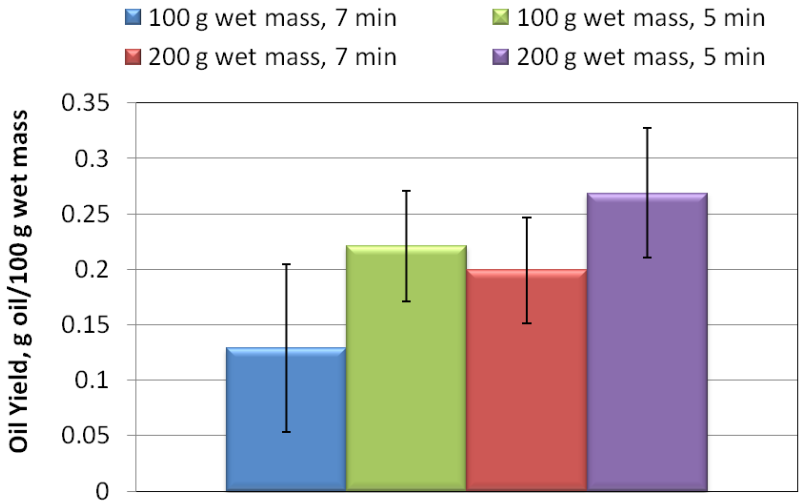


Figure 5: Average oil yield per 100 g wet mass for extracted grand fir samples. Process variations obscured any trends; oil yield was not affected by mass or extraction time. Error bars represent 90% confidence.

ANOVA analysis showed that oil yield did not correlate with specific extraction times or initial biomass weight because P-values were above 0.05 for all interactions. The 200 g sample ran for seven minutes showed a decreased trend in yield similar to the combined juniper sample; however, changes in yield were within normal variance. Comprehensive ANOVA results are given in Appendix A1-A3.

Observations

High process variation, low oil yields, and small scale obscured process trends. The research group noticed that extraction yield consistently decreased when the ice core melted before the 15-minute cooling period was finished. Qualitatively, the team believed, more finely ground material produced better oil yields; different grind sizes could not be analyzed in this study. Low extraction volumes made assessing oil yields difficult since a small amount of water remained in the measured vials. Changing extraction time and initial plant mass did not greatly affect yield; consumers may want to extract the greatest volume to decrease run cycles.

Gas Chromatography and Mass Spectrometry Analysis

Gas chromatography peaks were analyzed using mass spectrometry to determine the essential oil composition from SFME. Figure 6A-C shows the GC readouts for juniper berries, juniper needles, and grand fir needles, respectively. The x-axis represents retention time, t_r, in minutes while the y-axis represent the strength of the sensor response.

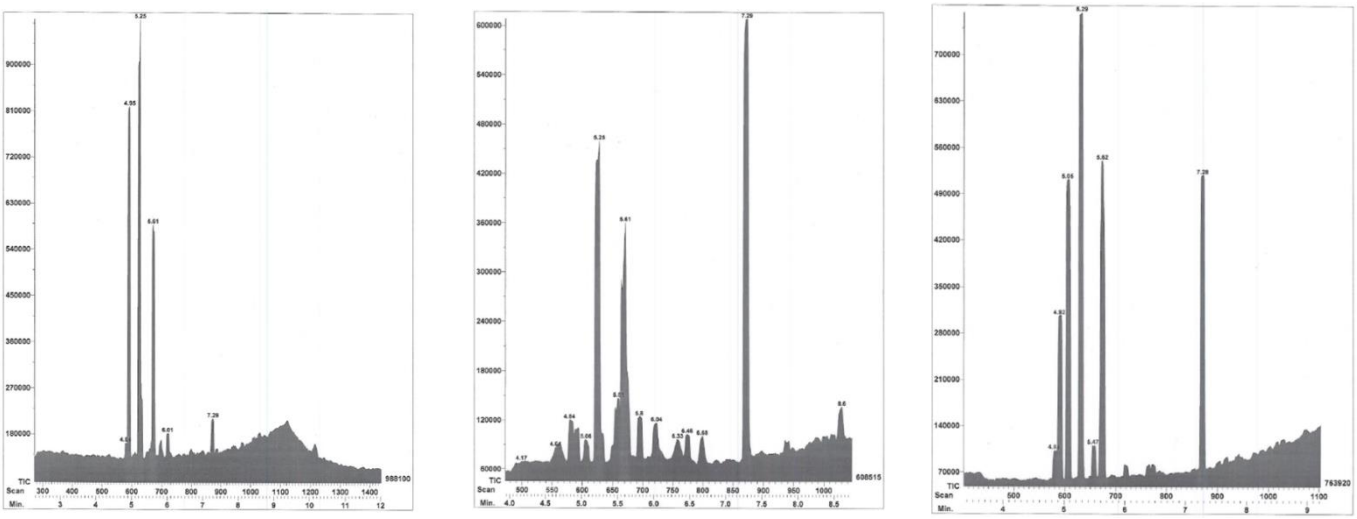


Figure 6: (A) GC readout for juniper berries. Three primary peaks were identified at retention times of 4.95, 5.25, and 5.61 minutes while another smaller peak was identified at t_r 7.29. Juniper berries contained the fewest number of peaks. **(B)** GC readout for juniper needles. Nine peaks were identified; juniper needles contained a more complex essential oil profile than juniper berries. **(C)** GC readout for grand fir needles where six primary peaks were identified.

Table 4 shows the tentative identification of the essential oil components at respective retention times for the GC readouts for the juniper berries and juniper needles, and grand fir needles, respectively. Standards must be purchased and run through the GC column to definitively assess components.

Juniper Berry GC-MS Peak Analysis		
Sample	Retention Time	Compounds
JBMS01	4.95	a-Pinene
JBMS01	5.25	a-Phellandrene
JBMS01	5.61	D-Limonene
JBMS01	7.29	Bornyl Acetate

Juniper Needles GC-MS Peak Analysis		
Sample	Retention Time	Compound
JNMS01	4.9	4-Carene
JNMS01	5.06	Camphene
JNMS01	5.25	a-Phellandrene
JNMS01	5.61	Lyratyl Acetate
JNMS01	5.8	4-Carene
JNMS01	6.04	cyclohexene
JNMS01	6.46	Bicyclo-trimethyl
JNMS01	6.68	cis-a-terpineol
JNMS01	7.29	Bornyl Acetate

Grand Fir GC-MS Peak Analysis		
Sample	Retention Time	Compounds
GFMS01	4.92	1S-a-Pinene
GFMS01	5.05	Camphene
GFMS01	5.29	a-Pinene
GFMS01	5.47	4-Carene
GFMS01	5.62	a-Phellandrene
GFMS01	7.28	Bornyl Acetate

Table 4: The identified compounds for specified GC retention times for juniper berries and needles, and grand fir needles. Preliminary identification must be verified by running standard solutions through the GC column.

Juniper berry oil composition differed from juniper needles, possibly contributing to the difference in essential oil scent. Grand fir contained a different essential oil profile than either juniper sample, demonstrating why the scents between the two plants differ. Detailed GC-MS readouts are presented in Appendix A6

Future Projects

GC-MS comparison of steam distillation and microwave extraction
A detailed comparison of essential oil profiles for steam distillation and SFME could be studied using GC-MS. Certain reactions can take place in the presence of microwaves or certain compounds can degrade through long exposure to heat. Oils extracted using the EssenEx-100 can be compared to published literature on similar essential oils extracted using steam distillation. Aromatic hydrosols can also be analyzed. GC-MS tests can be done in collaboration with the agricultural science department at Oregon State University.[10]

New plant materials
Many types of plant material have not been tested with the EssenEx-100 extraction unit. Potential experimental plant materials: false cedars, yew, etc.

Large scale/continuous microwave extraction process.
The major limiting factor for the EssenEx-100 unit is the small scale and condenser capability. A microwave and extraction unit fitted with an external condenser will have the capability for long continuous process and with large initial plant mass to yield appreciable oil volume.

Better separation technique
A new oil-water could be developed. The current project experienced high variability because the entire oil layer could not be separated from the water. Increased precision in separation process can reduce variability between operators and may aid consumers using the EssenEx-100.

Acknowledgements

Thank you to Dr. Hackleman, Jonathan Lebsack, and the OilExTech Company for guidance and for sponsoring the project. Thank you to Dr. Joe Karchesy, Dr. David Smith, and Dr. Edwin Jensen for providing vital knowledge on essential oils and the tree species under study. Thank you to Jeff Morre and Michelle Romero for assisting with GC-MS analysis. Thank you to Cory Schudel and the Holiday Tree Farm for providing grand fir samples; RR-Bar Ranch for providing western juniper samples; the Carkhuff, Reeker, and Bailey families, Clayton Tyler, and Matthew Blake for collecting western juniper samples; Lea Clayton for her assistance in ordering units essential to the project. Thank you lastly to Dr. Philip Harding for providing an amazing learning experience.

Appendix

Table A1: Detailed ANOVA analysis of grand fir. P-values indicate oil yield do not correlate to extraction time or initial plant mass.

ANOVA Group Grand Fir: constant mass, changing time			
Source of Variation	***F***	***P-value***	***F crit***
Between Groups @ (100 grams)	4.20	0.06	4.74
Between Groups @ (200 grams)	2.31	0.17	5.32

ANOVA Grpup Grand Fir: constant time, changing mass			
Source of Variation	***F***	***P-value***	***F crit***
Between Groups @ (5 min)	1.52	0.24	4.84
Between Groups @ (7 grams)	2.31	1.67	5.32

Table A2: Detailed ANOVA analysis of juniper needles and berries. P-values indicate oil yield is dependent on extraction time at 200 g. All other interactions were insignificant.

2-factor ANOVA with replicates (operators grouped)			
Source of Variation	***F***	***P-value***	***F crit***
Sample (100, 200 grams)	0.70	0.41	4.15
Columns (5, 7 min.)	3.21	0.08	4.15
Interaction	2.10	0.16	4.15

single-factor ANOVA (operators grouped): constant time, changing mass			
Source of Variation	***F***	***P-value***	***F crit***
Between Groups @ (5 min)	0.20	0.66	4.49
Between Groups @ (7 min)	2.49	0.13	4.49

single-factor ANOVA (operators grouped): constant mass, changing time			
Source of Variation	***F***	***P-value***	***F crit***
Between Groups @ (100 grams)	0.04	0.84	4.49
Between Groups @ (200 grams)	8.95	0.01	4.49

Table A3: ANOVA analysis showed higher oil yield for berries were attributed to plant type.

single-factor ANOVA (operators grouped): JN and JB			
Source of Variation	***F***	***P-value***	***F crit***
Berries and Needles Separate	27.42	0.006	7.71

Table A4: Compounds found in essential oils of grand fir needles (*Abies grandis*), western juniper needles (*Juniperus occientalis*), and berries from common juniper (*Juniperus communis*). Essential oils were extracted through traditional steam distillation. Common and western juniper berries are expected to have similar compounds since they belong to the same genus.

Abies grandis (needles)	*Juniperus occientalis* (needles)	*Juniperus communis* (berries)	
β-Pinene	α-Pinene	α -Thujene	Linalool
α-Pinene	Sabinene	α -Pinene	*Trans* -Sabinene hydrate
Camphene	Δ3-Carene	Camphene	Borneol
Tricyclene	Myrcne	Sabinene	Terpinene-4-ol
Myrcene	d-limonene	α -Pinene	α -Terpineol
Limonene	α-Terpinene	Myrcene	β -Cubebene
β-Phellandrene	p-cymene	α-Terpinene	β-Caryophyllene
Terpinolene	Camphor	p-Cymene	α -Humulene
Camphor	$C_{15}OH$	Limonene	Germacrene D
Bornyl acetate	C_{15}	1,8-cineol	γ -Cadinene
		γ-Terpinene	