# Comparative characterization of extractives in Alaskan Yellow, Eastern Red, and Western Red Cedars

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## Abstract
Softwoods, more specifically Cedars, are a set of tree species known to have extractive components with many different biological activities. Research has shown that certain compounds in Cedars are able to resist various forms of attack (microbial, fungal, insect, etc.). *Juniperus virginiana* (Eastern Red Cedar, *Cupressus/Chamacyparis nootkatensis* (Alaskan Yellow Cedar, & *Thuja plicata* (Western Red Cedar) are commonly studied. Eastern Red is ranked highest above Western Red and Alaskan Yellow in durability, which is a measure of the wood's resistance to attack. Cedars in particular often have high concentrations of cyclic compounds which exhibit insecticidal properties. Liquid-liquid fractionation is the analytical technique used to separate the chemicals with similar polarities. The fractionations were characterized by GC/MS. Through the analyses, all three species, revealed a number of mono- and bi-cyclic terpenes that may contribute to the durability of the tree.

**Keywords:** extractives, liquid-liquid fractionation (LLF), Gas Chromatography / Mass Spectrometry (GC/MS), polar / non-polar compounds, separation

## Introduction
Cedars are classified as softwoods which contain extractives known to resist xenobiotic and microbial attacks.[1] *Juniperus virginiana* (Eastern Red Cedar - ERC), *Cupressus/Chamacyparis nootkatensis* (Alaskan Yellow Cedar - AYC), & *Thuja plicata* (Western Red Cedar - WRC) are commonly studied Cedars. Softwoods, in general, are known to contain multiple aromatic compounds while Cedars, more specifically, have extractives rich with sterols and resin acids.[2]

Generally, softwoods have fewer polar compounds than hardwoods and also tend to be less durable[3]. Although softwoods are generally less durable than hardwoods, a number of Cedars are still considered durable and their extractives may exhibit biological activity. Literature has showed that ERC ranked the highest in durability while WRC and AYC fell further towards the middle of the list of durability.[4]

Cedars are also known for having many commercial uses. For example, the essential oils of Red Cedars (ERC & WRC) are rich with terpenes that are stable in various environments.[5] ERC has two main components, Widdrol & Cedrol that are both effective insecticide and anti-termitic products.[6] WRC is known to contain β and γ thujaplicin, terpenes with strong antifungal properties.[7,8] AYC also has two main components of this variety: Carvacrol and Nootkatene.[9,10] Carvacrol has been shown to exhibit strong antimicrobial and insecticidal properties[11] while other extractives from AYC have been shown to be highly resistant to termites and fungus.[9]

Liquid-Liquid Fractionation (LLF) was utilized to separate the chemical extracts with similar polarities in different fractions. Gas Chromatography / Mass Spectrometry (GC/MS) tools analyzed the fractions and identified and classified any similarities. The goals were to compare various chemical components of Cedar species' extractives and identify any similarities among them.

## Experimental
### Sample Preparation
Lumber samples of AYC, ERC, and WRC were collected and Wiley milled to 40 mesh. A soxhlet extraction with 9:1 acetone: water was run for 10 hours on each individual wood samples. The solvent extracts were dried with a rotary evaporator and placed in the freezer.

### Liquid-Liquid Fractionation
Solvent solutions of acetone and hexane were prepared in the following ratios (acetone: hexane): 10:90, 20:80, 30:70, 45:55, 60:40, 80:20, and 100:0. Approximately 100 mg of each wood extractives sample was placed

in a separate 3 mL vial and washed with each ratio of solvent to pure acetone. The samples were washed with 9 mL of each solution. During each wash, the vial was sonicated, vortex mixed, and allowed time for the solids to settle. The excess solution was decanted into a separate 7 mL vial and dried with nitrogen. Three replicates for each sample.

### Gas Chromatography/Mass Spectroscopy

Analysis of the extractives was performed using an HP 5890 Series II Plus Gas Chromatography/Mass Spectroscopy instrument equip with HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). Column temperature was initially 60 °C for 1 min, then gradually increased to 300 °C at 10 °C/min, and kept there for 3 min. Analytes were dissolved in 500-1000 µL of 1:1 (v/v) acetone: methanol, depending on weights, then pipetted into 1000 µL GC vials. Vials of dissolved sample were injected automatically into the instrument

## Results and Discussion

Since the cedars are in the same family we expected to find similar chemicals among the wood extractives. However, the compounds of interest are low in concentration and were not identified. The cedars characteristic were displayed in the figures by colorimetry, polarity and GC spectra of cedars major components.

All three Cedar species supernatants becomes darker as the polar solvent increased in the solvent solutions. This indicates polar compounds are dark in color, as shown in Figure 1.

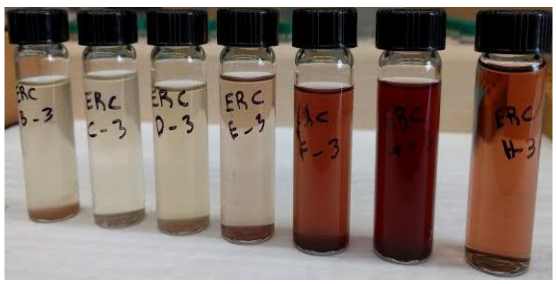
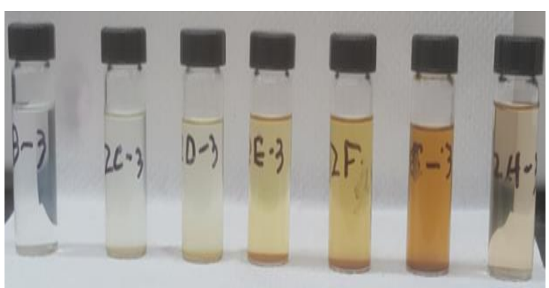
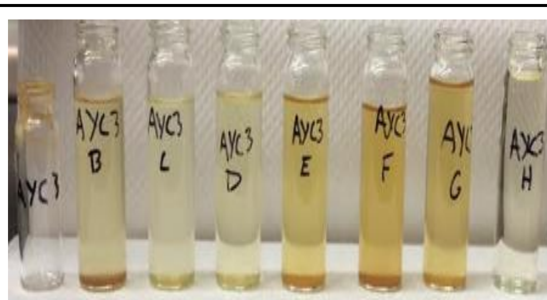
| | |
|---|---|
| ERC |  |
| WRC |  |
| AYC |  |

**Figure 1.** Vials of the solvent washes ratios (acetone: hexane): (b) 10:90, (c) 20:80, (d) 30:70, (e) 45:55, (f) 60:40, (g) 80:20, (h) 100:0 for ERC, WRC, and AYC.

In Figure 2, AYC and ERC weight percentages started at ~53% and ~29% respectively. The concentration decrease to 12% or less and ERC continued to gradually increase as the solvent solution changed in polarities. Base on this trend, Cedars intense color in vial G-F is not proportional to the concentration but due to a specific chemicals.

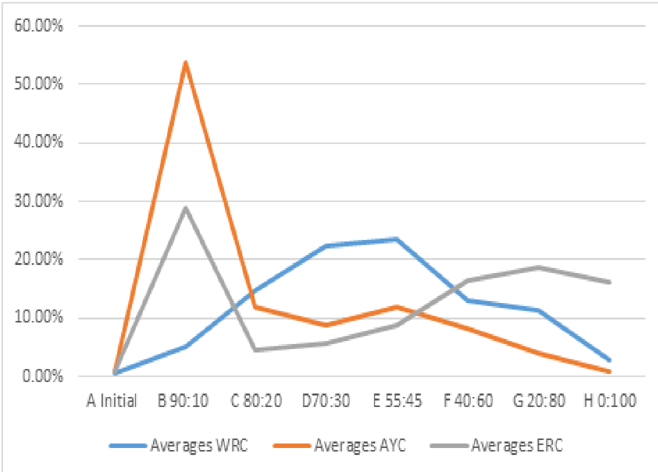



**Figure 2**. Weight Percentages of extractives collected by solvent fractions for WRC, AYC and ERC.

The the sum of weight percentages of extracts dissolved in polar, non-polar, and median phases are in Table 1. The B-D were added together and F-H were added together to create Table I. AYC contained the highest non-polar content (~75%) while WRC and ERC contained similar percentages of non-polar compounds (~40%). The value for ERC may vary due to the degradation of ERC. Kirker et al[4] determined that ERC is the most durable of the three woods tested while AYC is the least durable. Presence of polar compounds correlate with durability since ERC has the highest polar content and is the most durable while AYC contains the lowest percentage of polar compounds and ranks as the least durable of the three woods.[4] WRC had the largest amount of extracts dissolved in the median (vial E), indicating WRC has many compounds with an affinity for both polar and non-polar solvents. Values for the median do not appear to correlate with durability.

**Table 1**. Summation of weight percentages for polar, non-polar and median of wood extractives

| Wood Extractives | Non-Polar (B-D) | Polar (F-H) | Median (E) |
|---|---|---|---|
| **WRC** | 42.38% | 27.13% | 23.43% |
| **AYC** | 74.43% | 13.03% | 11.90% |
| **ERC** | 39.03% | 51.30% | 8.94% |

GC spectra show no similarities in terms of actual identical compounds. However, all three Cedars, as consistently stated in the literature, had similar classes of cyclic compounds, like terpenes.[5] Figure 3 are the chromatograms, spectra and structures of chemicals identified for AYC, WRC and ERC. The analyses showed all three Cedars appear to contain at least one major component with a cycloheptane structural base: Velleral in AYC, Nezukone in WRC, & Cedrol in ERC. (Velleral (AYC) and Nezukone (WRC) are not shown) However, due to how closely related the woods are, it seems likely that the three Cedars would share some identical compounds. It is likely that the compounds similar in these cedars are low in concentration, volatilized or too heavy to volatilize during GC-MS analyses. In the future, alternate analytical techniques, such as Liquid Chromatography, should be used to further characterize the components of these Cedar extractives.

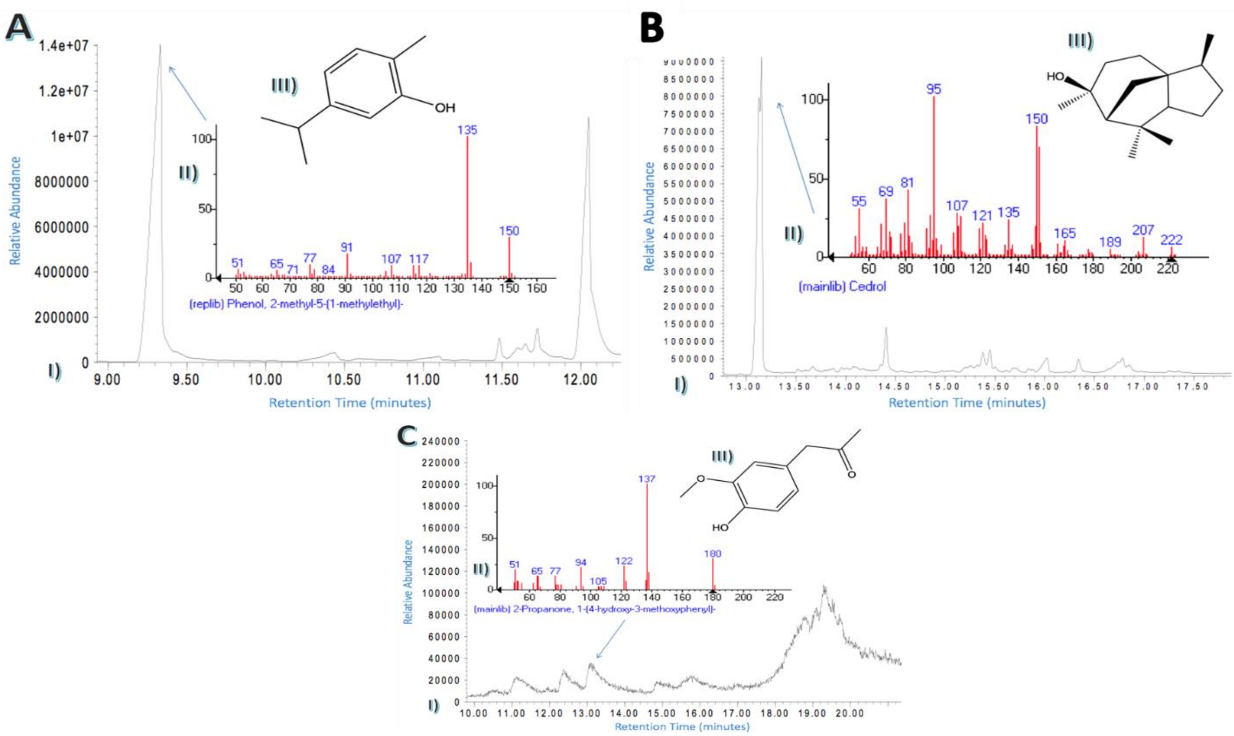



**Figure 3.** GC/MS analyses of A. Carvacrol (AYC) B. Cedrol (ERC) C. Thujaplicin (WRC) (I) Chromatogram (II) Spectra (III) Structure

## Conclusions

All three species, Alaskan Yellow Cedar, Western Red Cedar, & Eastern Red Cedar, differed in fractionation results and compounds characterized by GC-MS. Both ERC and AYC had similar trends in terms of dissolved extracts while WRC did not. GC-MS results showed that all three tree species showed many terpenes, consistently stated in the literature.[5] While each wood contained terpenes, none of them were identical despite all three woods being cedars. This suggests further analytical techniques are necessary to characterize these cedar extractives. It was also noticed that each of the three Cedar species contained different terpene compounds all with a cycloheptane base, a feature possibly characteristic of this type of tree.

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