

## Pressurized Fluids for Extraction of Cedarwood Oil from *Juniperus virginiana*

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The extraction of cedarwood oil (CWO) using liquid carbon dioxide (LC-CO<sub>2</sub>) was investigated and compared to supercritical fluid extraction, including the effects of extraction pressure and length of extraction. The chemical composition of the extracts was monitored over the course of the extraction as well. The cumulative yields of CWO from cedarwood chips using 80 L of carbon dioxide varied very little treatment to treatment, with all temperature/pressure combinations yielding between 3.55 and 3.88% CWO, and the cumulative yields were statistically equivalent. The rate of extraction was highest under the supercritical extraction conditions (i.e., 100 °C and 6000 psi). Under the liquid CO<sub>2</sub> conditions (i.e., 25 °C), the extraction rates did not vary significantly with extraction pressure. However, there were differences in the chemical composition of the collected CWO. Extractions at 100 °C gave a much lower ratio of cedrol/cedrene than extractions at 25 °C. The highest ratio of cedrol/cedrene was obtained using 25 °C and 1500 psi. The use of subcritical water was also investigated for the extraction of CWO as well. Although some CWO was extracted using this method, the temperature/pressure combinations that gave the highest weight percentage yields also gave oils with an off odor while those combinations that gave a higher quality oil had very low yields. It appears that the high temperatures and acidic conditions cause a dehydration of the tertiary alcohol, cedrol, to its hydrocarbon analogue, cedrene, during CO<sub>2</sub> or pressurized water extractions of cedarwood.

**KEYWORDS:** Carbon dioxide; cedarwood oil; cedrene; cedrol; dehydration; *Juniperus virginiana*; pressurized fluid extraction; water

### INTRODUCTION

Eastern red cedar, *Juniperus virginiana* L. (Cupressaceae), is widely distributed throughout the United States, and the range of this species has actually been increasing (1). Because this species often invades fields and rangelands, it is considered a pest species in states such as Texas, Oklahoma, and Nebraska (2–4). However, the wood from larger trees is used in the manufacture of chests and cabinets, and the sawdust and other waste wood from lumber mills is a source of cedarwood oil (CWO) (CAS no. 8000-27-9) (5). Some junipers are felled especially for their CWO as well as to improve range conditions (6). Although CWO is generally obtained by steam distillation (2), there are many potential benefits of using supercritical carbon dioxide (SC-CO<sub>2</sub>) instead. Eller and King (7) found that SC-CO<sub>2</sub> gave excellent yields of CWO, higher than previously reported for steam distillation. In addition, the CWO obtained by SC-CO<sub>2</sub> extraction more closely resembled the odor of the original wood than did CWO obtained by steam distillation. Although only supercritical conditions (i.e., temperatures and pressures above 31.1 °C and 1071 psi, respectively) were examined in this previous study, it was noted that CWO yields

were quite high even at the lowest temperature and pressure tested (i.e., 40 °C and 2750 psi, respectively). It was therefore hypothesized that liquid carbon dioxide (i.e., below 31.1 °C) might effectively extract CWO as well. In this study, we compared liquid CO<sub>2</sub> to supercritical CO<sub>2</sub> for the extraction of CWO. Both extraction rates as well as chemical profiles were examined. The use of pressurized water was also investigated as another environmentally friendly method to extract CWO.

### EXPERIMENTAL PROCEDURES

#### Liquid and Supercritical Carbon Dioxide Extraction of CWO.

Cedarwood chips were prepared from a kiln-dried cedar board purchased from a local lumber mill. A power wood planer was used to produce the chips/sawdust, and these were immediately packaged in a zipper-lock plastic bag, then wrapped in aluminum foil, and stored at –15 °C until used for extraction experiments (7).

Carbon dioxide extractions were conducted with an Spe-ed supercritical fluid extractor (SFE) (Applied Separations, Allentown, PA). About 10–12 g of cedarwood chips was weighed to the nearest 0.0001 g and then added to a 50 mL stainless steel extraction cell with glass fiber filter disks (18 mm dia) on the top and bottom of the cell. Four temperature/pressure combinations were evaluated. The liquid CO<sub>2</sub> extractions were conducted at 25 °C and pressures of 1500, 2750, and 6000 psi. The supercritical CO<sub>2</sub> extractions were conducted at 100 °C

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and a pressure of 6000 psi for comparison. There was a 10 min static hold followed by a dynamic extraction at a rate of ca. 2.5 L expanded CO<sub>2</sub> per minute. The variable restrictor was heated to 70 °C, and extracts were collected every 10 L of CO<sub>2</sub> up to 80 L in 12 mL glass vials. SFE/supercritical fluid chromatography (SFC) grade CO<sub>2</sub> (Air Products and Chemicals, Inc., Allentown, PA) was used for all extractions. Three replications of each temperature/pressure combination were performed.

Water was separated from the extracts as described by Eller and King (7), and the weight of the dry CWO was determined and the percent CWO extracted calculated based on the original mass of the wood chips (7). Solutions of CWO in hexane (ca. 300 ng/μL) were analyzed by gas chromatography (GC) to determine the percentage contribution of individual components (7). The masses of cedrene and cedrol were calculated from the total mass collected and the area percentages from the GC.

**Pressurized Water Extraction of CWO.** For the pressurized water study, the chips used were from a similar but different board than used for the CO<sub>2</sub> extraction experiments described earlier. The chips were prepared using a wood-boring drill bit and packaged in a zipper-lock plastic bag, wrapped in aluminum foil, and stored at -15 °C until used for extraction experiments.

Subcritical water extractions of cedarwood shavings were performed with an ASE 200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA) with pure (18 MΩ) water delivered by an ASE 200 solvent controller. An 11 mL ASE extraction cell was used for all experiments. A cellulose fiber was pressed into the extraction cell before the cell was filled with cedarwood shavings (ca. 1.0 g). The subcritical water extractions were performed with all possible combinations of 50, 100, 150, and 200 °C at 500, 750, 1500, and 3000 psi. All extractions utilized a 0 min preheat step, a 5 min heating period, three 5 min static extractions cycles, a 50% flush volume (the flush volume is defined as a percentage of the total cell volume), and a 1 min purge time. Two replications of each temperature/pressure combination were performed.

The aqueous extracts were extracted twice with 10 mL of diethyl ether. The extracts were centrifuged (5 min) to separate the aqueous and ether phases, and the ether layer was pipetted off, combined, and dried over sodium sulfate. This solution was then filtered through glass wool and concentrated under nitrogen at room temperature to yield a constant weight. The weight of the dry CWO was determined, and the percent CWO extracted was calculated based on the original mass of the wood chips.

Because the pressurized water extracts contained late-eluting, apparently high molecular weight or polar compounds, these extracts were not analyzed by GC but by SFC instead. A Lee Scientific Series model 600 chromatograph (Dionex Corp.) equipped with a flame ionization detector held at 350 °C was used. A Dionex SB-Phenyl-50 capillary column (10 m × 100 μm i.d., 0.5 μm film thickness), held at 50 °C, was used with the following pressure gradient: 1100 psi held for 5 min, then raised 14.7 psi/min to 1543 psi, and then raised 147 psi/min to 4474 psi. Injection of the samples was accomplished by a timed split automatic injector with a Valco valve (Valco, Inc., Houston, TX) for 0.5 s with a 200 nL loop. SFE/SFC grade CO<sub>2</sub> was the carrier fluid. Chromatograms were analyzed with a Data Jet integrator (Spectra-Physics Analytical, San Jose, CA).

**Statistical Analyses.** Analyses of variance (ANOVA) were conducted on data using Statistix 7 software (Analytical Software, Tallahassee, FL), and means were compared using least significant difference test at the  $P = 0.05$  level.

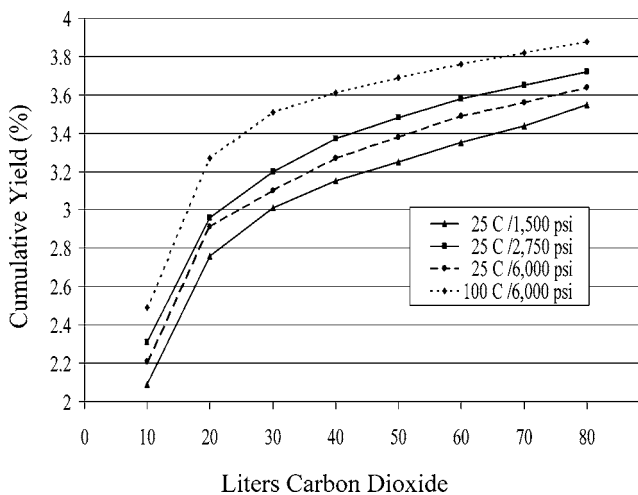
## RESULTS AND DISCUSSION

**Liquid and Supercritical Carbon Dioxide Extraction of CWO.** All of the treatments gave extracts that were yellow to amber in color, and all had a pleasant odor similar to that of the original wood chips. The overall recoveries of CWO for the liquid and supercritical fluid CO<sub>2</sub> extractions of the cedar chips are shown in Table 1. When a total of 80 L of CO<sub>2</sub> was used to extract ca. 10 g of chips, the percent yields varied only slightly between the various temperature/pressure combinations. In fact, the range was only from 3.55 to 3.88% recovery. After

**Table 1.** Overall Extraction of CWO Using Pressurized Carbon Dioxide<sup>a</sup>

temp (°C)	pressure (psi)	yield (%)	cedrene (mg)	cedrol	cedrol/cedrene ratio
25	1500	3.55 a	144.4 a	1914.9 a	13.25 b
25	2750	3.79 a	174.0 b	2250.1 a	12.93 b
25	6000	3.64 a	165.4 ab	2180.1 a	13.19 b
100	6000	3.88 a	187.8 b	2140.7 a	11.40 a

<sup>a</sup> Means without letters in common differ significantly (least significant difference).

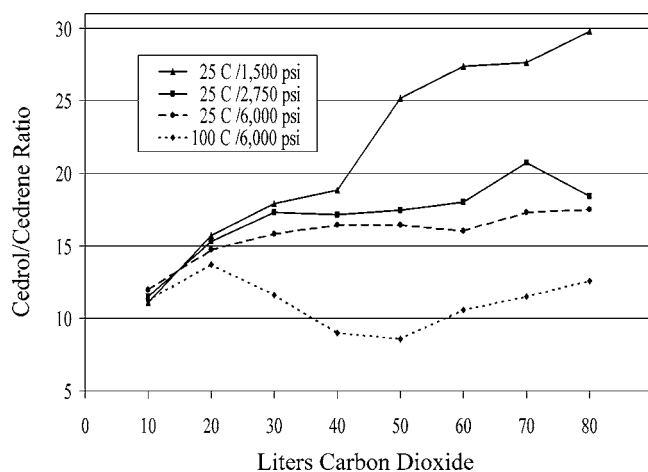


**Figure 1.** Cumulative yield of CWO as a function of extraction pressure, temperature, and liters of carbon dioxide.

80 L of carbon dioxide, there were no significant differences in total yields between any of the treatments ( $F_{3,8} = 1.35$ ,  $P = 0.32$ ). Interestingly, the yield at the relatively low pressure of 2750 psi at 25 °C was almost as high as the yield at 6000 psi and 100 °C.

The cumulative yields of CWO as a function of liters of CO<sub>2</sub> are shown in Figure 1. Although the overall yields, after 80 L, for the various temperature/pressure combinations were quite similar, the rate of extraction varied somewhat between the treatments. The extraction rate was highest for the combination of 6000 psi and 100 °C. Although the rate of extraction was lowest for the combination of 1500 psi and 25 °C, there was no clear relationship between extraction rate and pressure at 25 °C. Although there was no significant difference between treatments in CWO yield after 80 L, at 30 L, the yield for 6000 psi and 100 °C was significantly higher than the yield for 1500 and 6000 psi at 25 °C but not 2750 psi and 25 °C. However, at 30 L, there were no significant differences between any pressures in combination with 25 °C. Although our data indicate that relatively low temperature/pressure CO<sub>2</sub> combinations can be used to effectively extract CWO, there is a tradeoff between temperature/pressure and rate of extraction. However, because the capital costs of performing carbon dioxide extractions are proportional to the pressure and temperature of the extractions, extractions utilizing low pressures and temperatures would be less costly to conduct.

The results of the GC analyses indicated that essentially all of the fractions from all of the temperature/pressure combinations contained the major components previously reported for CWO, namely,  $\alpha$ - and  $\beta$ -cedrene, thujopsene, cuparene, cedrol, and widdrol (8). The most abundant component was cedrol. The ratio of the tertiary alcohol, cedrol, to its hydrocarbon analogue, cedrene, was used for comparing the composition of the CWOs



**Figure 2.** Ratio of cedrol to cedrene as a function of extraction pressure, temperature, and liters of carbon dioxide.

obtained from the various treatments. The ratios of cedrol/cedrene for the various temperature/pressure combinations as a function of liters of CO<sub>2</sub> are shown in **Figure 2**. All of the treatments had similar ratios at the first fraction collected at 10 L, i.e., ca. 12:1. However, as the extraction proceeded, the ratio of cedrol/cedrene began to vary with the various temperature/pressure combinations. At the lowest pressure (i.e., 1500 psi) and 25 °C, the ratio of cedrol/cedrene rose steeply and was ca. 30:1 after 80 L. Conversely, at the supercritical conditions of 100 °C and 6000 psi, the ratio of cedrol/cedrene rose only slightly and then decreased to ca. 8:1 before increasing back to ca. 12:1. The pressures of 2750 and 6000 in combination with 25 °C rose slightly from ca. 12:1 up to ca. 17:1. The total amounts of cedrene and cedrol collected over the 80 L were calculated from the masses collected from each fraction and the relative percentages of these compounds. The total masses of cedrene and cedrol for the four temperature/pressure combinations are shown in **Table 1**. Although there was no significant difference between treatments in the amount of cedrol collected ( $F_{3,4} = 3.9$ ,  $P = 0.11$ ), there were some significant differences in the amounts of cedrene collected ( $F_{3,4} = 8.9$ ,  $P = 0.03$ ). The highest amount of cedrene was from the combination of 100 °C and 6000 psi. In addition, there were also significant differences in the ratios of cedrol/cedrene as well ( $F_{3,4} = 45.9$ ,  $P = 0.001$ ). The ratio of cedrol/cedrene was lowest for the combination of 100 °C and 6000 psi, and the ratio of cedrol/cedrene for this combination was significantly lower than any of the pressures tested in combination with 25 °C. Although it is not certain whether this temperature difference is due to different rates of extraction for cedrene at the two temperatures tested, or possibly to chemical reaction occurring during the extraction, it is likely that at 100 °C, there is some dehydration of the tertiary alcohol, cedrol, to the hydrocarbon, cedrene. Koedam and Looman (9) discuss how the acidity of the water during steam distillation caused changes in CWO composition. Adams (10) discusses the effect of pH on the decomposition of CWO during steam distillations and reported that the pH changed from 7.12 at the start of the hydrodistillation of cedarwood to 6.17 after 2 h. Pickett et al. (11) also reported that the acidic conditions present during steam distillation are responsible for the degradation of the tertiary alcohol, linalool. In addition to the acidity due to the wood, the presence of the carbon dioxide during the supercritical extractions may produce carbonic acid leading to a decreased pH as well. Toews et al. (12) reported that the pH of water in equilibrium with CO<sub>2</sub> decreases as the pressure increases and increases as the

**Table 2.** Mean Yield and Composition of CWO Obtained by Water Extraction

temp (°C)	pressure (psi)	yield (%)	cedrene (%)	cedrol (%)	cedrol/cedrene ratio
50	500	0.34	0.6	14.6	24.3
50	750	1.10	0.1	8.4	84.0
50	1500	0.55	0.6	7.4	12.3
50	3000	0.47	0.2	2.6	13.0
100	500	0.66	1.6	23.9	14.9
100	750	0.91	3.4	23.8	7.0
100	1500	0.56	2.3	30.7	13.3
100	3000	0.54	3.2	27.0	8.4
150	500	1.10	19.4	3.6	0.2
150	750	1.13	17.7	2.5	0.1
150	1500	1.05	27.2	5.2	0.2
150	3000	1.05	17.6	5.2	0.3
200	500	4.04	18.3	2.7	0.1
200	750	3.7	8.2	4.1	0.5
200	1500	3.34	14.9	4.0	0.3
200	3000	3.85	4.3	5.2	1.2

temperature increases; however, the pH varied only from 2.80 to 2.95 over the range of pressures (1029–2939 psi) and temperatures (25–70 °C) tested. It is likely that the CO<sub>2</sub> in our extractions caused acidic conditions leading to the conversion of cedrol to cedrene, especially at the higher temperatures. Although there are some differences in the chemical composition of the CWO between the collected fractions, they are relatively minor and would have little effect on the overall composition of the total CWO collected nor are the differences sufficient to be used to efficiently enrich fractions in individual components of CWO. However, the effects of pH should not be ignored and should be considered if one component such as cedrol or cedrene is more desirous.

**Pressurized Water Extraction of CWO.** The recovery of CWO from the cedar chips using pressurized water is shown in **Table 2**. Although there was a clear trend of CWO yields increasing with higher temperatures, there was very little apparent effect of pressure on yield. The ANOVA of the yield data indicated that there were significant main effects of pressure ( $F_{3,21} = 3.9$ ,  $P = 0.02$ ) and temperature ( $F_{3,21} = 405.2$ ,  $P < 0.0001$ ) but no significant pressure by temperature interaction ( $F_{9,21} = 2.2$ ,  $P = 0.07$ ). The overall yields (i.e., summed over temperature) for 500, 750, 1500, and 3000 psi were as follows: 1.53, 1.72, 1.37, and 1.48%, respectively. The overall yield at 750 psi was significantly higher than the yields obtained using 1500 or 3000 psi; all other pairs were statistically equivalent. The overall yields (i.e., summed over pressure) for 50, 100, 150, and 200 °C were as follows: 0.62, 0.67, 1.08, and 3.74%, respectively. The overall yields obtained at 50 and 100 °C were statistically equivalent; however, all other pairs were significantly different. At a temperature of 50 °C, the yields of CWO were fairly low and ranged from about 0.4 to just over 1%. At 200 °C, the yields were fairly high, at approximately 4%. These same chips were extracted using supercritical CO<sub>2</sub> at a temperature of 100 °C and a pressure of 4000 psi (80 L expanded CO<sub>2</sub>) for comparison of yields between these two methods. Using these conditions, the CO<sub>2</sub> gave a yield of 2.9%. This is greater than the recoveries of CWO using water at temperatures of 150 °C or less but less than the yield using water at temperatures of 200 °C. It is likely that the 200 °C water extracted materials other than just CWO, which would account for the higher than expected yield for this treatment. The SFC analyses indicated that there were unidentified compounds, which eluted late from the column and were presumably more polar or higher molecular weight compounds.

The CWO samples from the extractions done at the lower temperatures (i.e., 100 °C or less) had an odor similar to that of the original wood chips as well as the CO<sub>2</sub> extracts. However, the CWO samples from the extractions done at the higher temperatures (i.e., 150 °C or higher) were very dark and had a slightly different odor with a somewhat "burnt" characteristic, suggesting that these high temperatures may cause some degradation of the CWO. The SFC analyses indicated that this may indeed be the case. The chemical analyses of the CWO obtained using the lower temperatures contained the three most abundant components of CWO, namely, cedrene, thujopsene, and cedrol, in ratios similar to those seen in the CWO obtained by CO<sub>2</sub> extraction. However, the CWO obtained using the higher temperatures contained almost no cedrol and higher amounts of cedrene than that seen for treatments using lower temperatures (Table 2). The ratio of cedrol/cedrene was very high at 50 °C and 500 or 750 psi. In addition, the ratio of cedrol/cedrene was also high at 50 °C and 1500 or 3000 psi as well as at all pressures at 100 °C. At temperatures of 150 or 200 °C, the ratio of cedrol/cedrene was very low. As discussed previously, it is likely that cedrol is dehydrated to cedrene during CO<sub>2</sub> extraction and this same reaction may be responsible for the changes seen in the amounts of these two compounds in the samples of CWO collected using the highest temperatures of pressurized water. This hypothesis was tested by dissolving 50 mg of cedrol in 1 mL of hexane and placing this solution on glass wool. The hexane was allowed to evaporate, and the glass wool was subsequently extracted using water at 150 °C and 1500 psi as described above. The collected extract was analyzed by SFC and found to contain cedrol but no cedrene. However, when a similar solution of cedrol in hexane was placed on cedarwood chips previously extracted by supercritical CO<sub>2</sub> (100 °C, 4,000 psi, 80 L) to remove all CWO and subsequently extracted using water at 150 °C and 1500 psi, the collected extract contained very little cedrol and a large amount of cedrene, almost 10 times as much cedrene as cedrol. Water extracts of the chips previously extracted by supercritical CO<sub>2</sub> without added cedrol contained virtually no cedrol or cedrene. Therefore, it appears that cedrol is dehydrated to cedrene under high temperature conditions and that acidic properties of the wood catalyze this conversion.

Although it is possible to use pressurized water to extract CWO from cedar chips, the temperatures that gave the highest yields gave low quality oil. Conversely, the temperatures that gave a higher quality oil had very low extraction rates.

In conclusion, liquid carbon dioxide has been shown to be an effective means for extracting CWO. In addition, the lower temperatures associated with the use of liquid CO<sub>2</sub> also seem to avoid the dehydration of cedrol to cedrene during the course of the extraction, especially under acidic conditions. Because extractions utilizing liquid CO<sub>2</sub> do not require the high pressures and temperatures associated with supercritical fluid extractions, the capital costs associated with liquid CO<sub>2</sub> extractions are significantly less than those for supercritical fluid extractions.

CWO has great potential as use as a safe natural bioactive agent, including wood preservation against both insects (13–15) and microbes (16), insecticides (17) and acaricides (18), and as mosquito (19) and cockroach repellents (20). Such uses of CWO extracted by carbon dioxide are currently under investigation. The development of such uses as these may provide an additional source of income for rural families in addition to the benefit of the removal of invasive junipers.

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## LITERATURE CITED

- (1) Schmidt, T. L.; Leatherby, E. C. Expansion of eastern red cedar in the lower midwest. *Northern J. Appl. For.* **1995**, *12*, 180–183.
- (2) Adams, R. P. Investigation of *Juniperus* species of the United States for new sources of cedarwood oil. *Econ. Bot.* **1987**, *41*, 48–54.
- (3) Wilson, J.; Schmidt, T. Controlling eastern redcedar on rangelands and pastures. *Rangelands* **1990**, *12*, 156–158.
- (4) Bidwell, T. G. Eastern red cedar ecology and management. *Oklahoma St. University Ext. Facts No. 2868*; 1993; Oklahoma State University: Stillwater, OK, 4 pp.
- (5) Arctander, S. *Perfume and Flavor Materials of Natural Origin*; Det. Hoffenbergske Etablissement: Denmark, 1960.
- (6) Lawless, J. *The Illustrated Encyclopedia of Essential Oils*; Barnes and Noble: New York, 1995.
- (7) Eller, F. J.; King, J. W. Supercritical carbon dioxide extraction of cedarwood oil: A study of extraction parameters and oil characteristics. *Phytochem. Anal.* **2000**, *11*, 226–231.
- (8) Adams, R. P. Analysis of juniper and other forest tree oil. *Mod. Methods Plant Anal.* **1991b**, *12*, 131–157.
- (9) Koedam, A.; Looman, A. Effect of pH during the distillation on the composition of the volatile oil from *Juniperus sabina*. *Planta Med. Suppl.* **1980**, 22–28.
- (10) Adams, R. P. Cedarwood oil: Analyses and properties. *Mod. Methods Plant Anal.* **1991a**, *12*, 159–173.
- (11) Pickett, J. A.; Coates, J.; Sharpe, F. R. Distortion of essential oil composition during isolation by steam distillation. *Chem. Ind.* **1975**, *5*, 571–572.
- (12) Toews, K. L.; Shroll, M.; Wai, C. M. pH-defining equilibrium between water and supercritical CO<sub>2</sub>. Influence on sfe of organics and metal chelates. *Anal. Chem.* **1995**, *67*, 4040–4043.
- (13) Carter, F. L. Responses of subterranean termites to wood extractives. *Mater. Org.* **1976**, *3*, 357–364.
- (14) Adams, R. P.; McDaniel, C. A.; Carter, F. L. Termiticidal activities in the heartwood, bark/sapwood and leaves of *Juniperus* species from the United States. *Biochem. Syst. Ecol.* **1988**, *16*, 453–456.
- (15) McDaniel, C. A.; Dunn, B. S. Can wood extractives be used as wood protectants. *Gen. Technol. Rep. Soc.* **1994**, *101*, 61–63.
- (16) Clark, A. M.; McChesney, J. D.; Adams, R. P. Antimicrobial properties of heartwood, bark/sapwood and leaves of *Juniperus* species. *Phytother. Res.* **1990**, *4*, 15–19.
- (17) Oda, J.; Ando, N.; Inouye, Y. Studies on insecticidal constituents of *Juniperus recurva* Buch. *Agric. Biol. Chem.* **1977**, *41*, 201–204.
- (18) Panella, N. A.; Karchesy, Maupin, G. O.; Malan, J. C. S.; Piesman, J. Susceptibility of immature *Ixodes scapularis* (Acari: Ixodidae) to plant-derived acaricides. *J. Med. Entomol.* **1997**, *34*, 340–345.
- (19) Curtis, C. F.; Lines, J. D.; Ijumba, J.; Callaghan, A.; Hill, N.; Karmimzad, M. A. The relative efficacy of repellents against mosquito vectors of disease. *Med. Vet. Entomol.* **1987**, *1*, 109–119.
- (20) Appel, A. G.; Mack, T. P. Repellency of milled aromatic eastern red cedar to domiciliary cockroaches (Dictyoptera: Blattellidae and Blattidae). *J. Econ. Entomol.* **1989**, *82*, 152–155.

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