TECHNICAL NOTE

Potential for Utilizing Western Juniper (*Juniperus occidentalis*) Biomass for Oil Extraction and as a Fermentation Medium

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Abstract

Steam-distilled essential oils from western juniper (*Juniperus occidentalis*) foliage and twigs were tested for activity against subterranean termites and fungi. Foliage residues were pretreated with dilute sulfuric acid and enzymatically treated to determine their digestibility. Essential oil recovery and sugar yields were higher for foliage than twigs. Foliage essential oil exhibited excellent antifungal and termiticidal activities at the concentrations tested. These results highlight the potential for integrating steam distillation of western juniper foliage to remove essential oil with enzymatic digestion of extraction residues. Additional studies to optimize distillation and pretreatment conditions for foliage are recommended.

Western juniper (*Juniperus occidentalis*) is native to the arid intermountain region of the western United States. Fire suppression over the past century has sharply reduced the incidence of range fires that formerly limited the growth of this species (Bedell et al. 1993, Coultrap et al. 2008). Juniper trees consume large quantities of scarce water and suppress the growth of other plants, resulting in a significant decline in rangeland quality in regions dominated by western juniper (Bedell et al. 1993). In response, the federal government has encouraged juniper removal; trees are often felled or pulled from the ground and left on site. Costs for these operations can be high—approaching US$500 to US$800 per acre (Young et al. 1982).

One approach for improving the economics of western juniper removal would be to identify products that could be produced from the biomass. The overall goal in this study was to improve western juniper biomass utilization by (1) determining the essential oil yields from both juniper twigs and foliage; (2) assessing the efficacy of these oils as natural biocides for termites and decay fungi; and (3) assessing potential use of extracted twig and foliage residues as a fermentation substrate for biofuels or other fermentation products.

Materials and Methods

Western juniper foliage and twigs (5 to 10 cm in diameter) were collected from trees near Sisters, Oregon, in the Deschutes National Forest. The two materials were stored at 5°C. Both materials were ground to pass a 20-mesh screen to help improve fluid access into the tissues.

Steam distillation

Foliage and twigs were separately subjected to steam distillation by adding 2 to 3 kg of a given material to a distillation column, which was attached to a 12-liter round-bottom flask containing 6 liters of distilled water. The system was operated until oil recovery had become negligible (approximately 6 to 8 h). Yield was measured as the ratio of the weight of the oil to the original wet weight of the material. The residual twigs and foliage were frozen for later use in the hydrolysis trials. Four separate extractions were performed with each material.

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Extract bioactivity

Oil bioactivity was assessed against brown and white rot fungi [*Postia placenta* (Fr.) M. Larsen et Lombard (Isolate Mad 698) and *Trametes versicolor* (L. ex. Fr.) Pilát (Isolate R. 105), respectively] and eastern subterranean termites (*Reticulitermes flavipes* L.). Ponderosa pine (*Pinus ponderosa* L.) and southern pine (*Pinus* sp.) sapwood blocks (20 by 15 by 5 mm long and 19 by 19 by 6 mm long, respectively) were oven dried (50°C) and weighed. The blocks were then impregnated with an excess of 95 percent ethanol solution containing 0, 10, 20, or 30 percent by volume of foliage extract (as discussed below, twig extract was not tested due to low yields) using a vacuum desiccator and left in the open for 48 hours before being conditioned to a constant weight at 23°C and 65 percent relative humidity. The blocks were then weighed to provide an initial weight for determining subsequent fungal associated weight loss. The ponderosa pine blocks were sterilized by exposure to 25 mrad of ionizing radiation from a cobalt 60 source. The blocks were incubated with brown or white rot fungus inoculum in plastic petri dishes containing 0.5 percent malt extract agar for 12 weeks at 28°C, six blocks per fungus. Weight loss over the test period was used as the measure of extract efficacy.

Southern pine blocks were used to evaluate resistance to *Reticulitermes flavipes* L. attack in a no-choice test following procedures described in American Wood Protection Association (AWPA) Standard E1 (AWPA 2010).

Hydrolysis

The potential for using the steam-distilled foliage or twigs as a fermentation substrate was evaluated by first subjecting the materials to a dilute sulfuric acid pretreatment and then digesting the pretreated material with enzymes. Acid pretreatments were conducted with three replicates per treatment at the following time and temperature conditions: 48 hours at room temperature, 1 hour at 121°C, or 3 hours at 121°C.

Enzymatic hydrolysis was carried out by digesting the pretreated material with Accellerase 1500 enzyme complex at 60 filter paper units per gram of cellulose loading. Two control assays were also run, one without juniper substrate and the other without the enzymes. Each treatment was run in triplicate. Reducing sugar yields from both acid hydrolysis and enzyme hydrolysis were measured by the dinitrosalicylic acid method according to procedures described by Adney and Baker (1996) using glucose as a standard.

Results and Discussion

Steam distillation

Oil yields from foliage were higher than those from twigs (0.77% ± 0.10% vs. 0.13% ± 0.05% yield, respectively). Foliage yields were slightly higher than those reported previously (Table 1). This was likely the result of tree-to-tree variability, seasonal differences, and different extraction times. Lower yields from twigs were likely due to the presence of bark and sapwood, both of which contain little if any essential oils. In addition, foliage produced greenish yellow oil with a strong burning odor, while twigs produced a pale yellow oil without the burning odor. Given the very low oil yields from twigs, bioactivity focused solely on foliage oils.

Extract bioactivity

Weight losses of ethanol-treated control ponderosa pine sapwood blocks exposed to *P. placenta* ranged from 22 to 26 percent, while *T. versicolor* produced negligible weight losses in the control blocks (Fig. 1). White rot fungi typically have difficulty attacking coniferous blocks, and these results confirm this characteristic. The brown rot weight losses indicated that the test fungus was causing substantial mass loss. Weight losses for blocks treated with dilutions of the juniper foliage extracts ranged from 1 to 3 percent, with the higher weight losses occurring at the highest oil concentration (Fig. 1). The slightly higher weight losses associated with higher concentrations of extract likely reflect leaching losses of chemical from the blocks during the decay test because the fungus was completely killed at this level and was inhibited at the 10 or 20 percent dilutions. These results indicate that the threshold for fungal protection was below the 10 percent dilution tested.

Juniper foliage oil–treated blocks exhibited exceptional activity against *R. flavipes* (Table 2). Weight losses of control blocks ranged from 28 to 36.9 percent, while treated blocks experienced decreasing weight losses with increasing foliage oil concentration. Complete termite mortality occurred in chambers with blocks treated with 20 or 30 percent oil, while 79 percent mortality occurred at the 10 percent dilution. Some slight attack occurred on the 20 and 30 percent dilution blocks, but the average ratings were still 9.2 and 9.6 percent, respectively; visual ratings varied from 0 to 4 for the control blocks. The results indicate that the foliage oil had excellent activity against both fungi and termites. These results are consistent with previous research (Adams et al. 1988, Clark et al. 1990).

Fermentation potential

Acid pretreatment of foliage material for 48 hours at room temperature produced negligible sugar release, while autoclaving for 1 hour increased levels nearly fourfold (Fig. 2). There was no evidence that additional heating (3 h) had a marked effect on sugar release. Sugar yields from acid pretreatment of twig material were all below detection limit of this test, suggesting that none of the pretreatment conditions solubilized carbohydrates in branchwood.

Enzymatic digestion of acid pretreated foliage resulted in release of 41 to 43 percent of the available substrate, with no

Table 1.—Yields of essential oils from western juniper from Oregon.

Source	Collection site	Month collected	Tree part	Distillation time (h)	% yields
Burnet (1954)	Prineville	Dec	Heartwood (top)	24	1.09
			Heartwood (butt)	24	2.26
Kurth and Ross (1954)	Prineville	May	Heartwood	4–6	1.4
Adams (1987)	Juntura	Feb	Heartwood	20	2.33
Yesenofski (1996)	Warm Springs	Nov	Heartwood	1–4	0.4
			Foliage	1–4	0.4

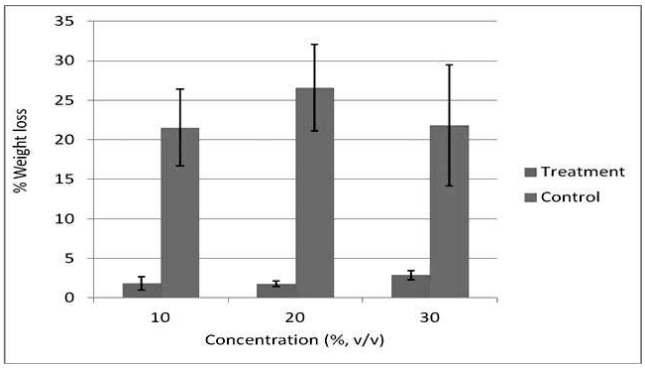

Figure 1.—Weight losses (%) of juniper foliage oil–treated wood blocks exposed to Postia placenta *in an agar block decay test. Error bars represent one standard deviation.*

Table 2.—Wood weight loss, termite mortality, and visual rating of blocks treated with 10, 20, or 30 percent juniper leaf oil and exposed to Reticulitermes flavipes *in an American Wood Protection Association standard E1 test.*[a]

Juniper oil concn. (%)	Wood wt loss (%)		Mortality (%)		Visual rating[b]	
	Control	Treated	Control	Treated	Control	Treated
0	28.36	—	0	—	4	—
10	36.85	10.56	12	79	3.2	8.4
20	33.48	6.90	48	100	3.2	9.2
30	32.46	5.25	32	100	0	9.6

[a] Values are means of five replicates.
[b] Values range from 10 (no evidence of damage) to 0 (complete failure).

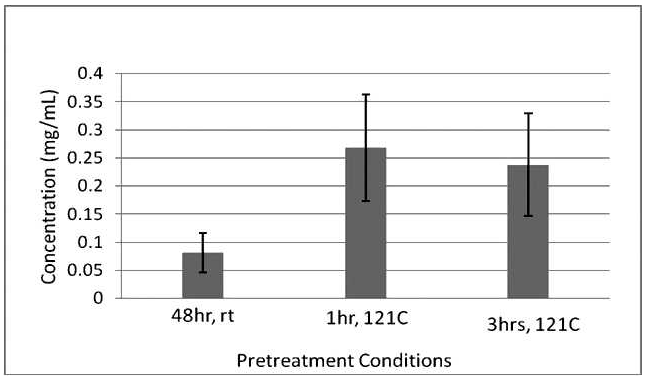

Figure 2.—Effect of sulfuric acid pretreatment conditions on reducing sugar yield for extracted juniper foliage. Values represent means of three replicates, while error bars represent one standard deviation.

evidence that longer heating produced increased release. The results do suggest that acid pretreatment of steam-distilled foliage produces a reasonable reducing sugar yield that might be suitable for fermentation. Sugar yields from enzymatic digestion of nontreated twig material were slightly higher than those from treated material, suggesting that extraction had removed water-soluble constituents including small amounts of sugars. A follow-up test performed to determine the contribution of steam distillation to improved substrate suitability to fermentation suggested that steam distillation negatively affected branchwood digestibility and positively affected foliage digestibility.

Generally, sugar yields from twig material were much lower than those from foliage material, suggesting that the twig material would be a poor fermentation substrate.

Conclusions

Juniper foliage produced a reasonable amount of oil that was highly effective against both wood decay fungi and subterranean termites. The extracted foliage was also useful for subsequent acid hydrolysis to create substrates for fermentation to produce ethanol. In contrast, both oil and sugar yields from branchwood were very low. These results suggest that additional value-added materials may be obtained from juniper foliage to help offset the removal costs.

Future research is recommended to explore the economics of the juniper foliage oil distillation, to determine the optimum concentration and retention of juniper foliage oil as a biocide, and to determine the optimum conditions for acid pretreatment of extracted foliage.

Acknowledgments

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