Final Report

Survey of Potential Antimicrobial Activity in Organic Extracts of Western Juniper

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Introduction

Markets are being developed for western juniper wood products both to partially defray the costs of rangeland habitat restoration and to increase jobs in the forest products industry. One promising product for western juniper is shavings for the equine and canine bedding markets. Shavings from several tree species are already used in the western U.S. including pine, fir and western red cedar. The question presented to our research group was ‘what benefit would western juniper have over the other established bedding products’?

The amount and duration of rainfall in the Pacific Northwest and Northern California presents an ideal environment for bacterial and fungal diseases in domestic and farm animals. One common disease is thrush of the frog in horses. The etiologic agent for thrush is *Fusobacterium necrophorum*. Infection by this anaerobe is typically followed by a fungal infection by yeasts such as *Candida albicans*. *Actinomyces bovis* is associated with contagious foot rot principally in sheep and cattle. *Clostridium perfringens* is commonly found in soil and enters animals through wounds to cause tissue necrosis in dogs and cattle. Development of these diseases is aided by lack of bedding in rainy months since animals are in contact with soil and mud.

Antimicrobial compounds provided to animals in their bedding may aid in reducing the incidence of these diseases during the long rainy months experienced in the Northwest. An investigation to determine whether the natural wood preservatives found in western juniper have antimicrobial activities against the widespread animal pathogens outlined above was undertaken. The objective of this work was to determine the activity of juniper heartwood extracts against bacteria and yeast compared to the effects of extracts from Alaska yellow cedar, western red cedar, pine and fir.

Materials & Methods

Bacteria and Media. All microorganisms were obtained from the American Type Culture Collection (Manassas VA) and maintained on slants in the respective medium. Anaerobic bacteria were manipulated in an anaerobic

glove bag (Coy Laboratory Products Inc., Grass lake MI) and media were prepared using the serum bottle technique. *Fusobacterium necrophorum* (ATCC 27852) was grown in a modified chopped meat medium (ATCC 1490) buffered with carbonate/CO^2. For MIC assays this medium was clarified through a 0.8 μ m filter. *Clostridium perfringens* (ATCC 43403) was grown in reinforced clostridium medium (Difco). *Actinomyces bovis* (ATCC 13683) was grown in Actinomyces broth (BBL). All anaerobic bacteria were grown at 38° C. *Candida albicans* was grown in Sabouraud dextrose broth (Difco) at 38° C. *Aspergillus flavus* (ATCC 52082) was grown in malt extract medium (Difco) at 30° C.

Plant Extracts. Heartwood oils of western juniper (*Juniperus occidentallis*) and Alaska yellow cedar (*Chamaecyparis nookatensis*) were prepared from fresh ground heartwood and steam distilled for 6 hr. Heartwood methanol extracts were prepared from western juniper, Douglas fir (*Pseudotsuga menziesii*), western red cedar (*Thuja plicata*) and Alaska yellow cedar. A methanol sapwood extract was prepared from ponderosa pine (*Pinus ponderosa*). Nookatin was crystallized from the steam distilled oil of Alaska yellow cedar. Alpha and beta cedrene and (+)-cedrol were obtained from Fluka (Ronkonoma NY).

MIC Technique. A standardized tube assay for the anaerobic microbes was developed. Twelve hour cultures were inoculated into 5 ml of the respective medium in Balch tubes to give a final concentration of 3×10^7 cells per ml. The extracts were dissolved in methanol:Tween 80 (4:1 v/v) and added to each tube of culture to give final concentrations as indicated in Table 1. The highest concentration tested was 2 mg/mL since it was not possible to dissolve higher concentrations. The tubes were incubated on their sides on a Model 3520 Lab-Line shaker (Melrose Park IL) at 250 rpm and 38° C. *Candida albicans* was incubated in culture tubes in an upright position under the same conditions. Growth of cultures was followed by absorbance at 600 nm on a Milton Roy Spectronic 20 spectrophotometer (Rochester NY) for 6.5 hours. Growth curves showed that all bacterial and yeast strains showed 5-fold increases in absorbance over this time period. Each microorganisms was tested for solvent inhibition to increasing concentrations of methanol:Tween-80 and this concentration was not exceeded in this study. The minimum inhibitory concentrations was defined as the concentration of extract that allowed no increase in absorbance at 600nm after 6.5 hours of incubation.

Results

Antimicrobial activity was assayed for the heartwood and sapwood methanol extracts as well as the essential oils. Western juniper methanol extract of heartwood showed antimicrobial activity against *F. necrophorum* and *C. perfringens* in the milligram per milliter range (Table 1) and against *A. bovis* in the part per million range. No activity was seen against *C. albicans* (yeast). The essential oil of western juniper showed activity against *C. perfringens* and *A. bovis* as well as *C. albicans*. The essential oil of Alaska yellow cedar showed a nearly 10-fold higher activity than the methanol extract of juniper heartwood or the essential oil. Western red cedar showed activity against *C. albicans* but not against any of the bacteria. Although western red cedar is commonly referred to as having antimicrobial activity, we could only substantiate antifungal activity in the scientific literature. Our results agree with the antifungal properties but we observed no antibacterial properties. Douglas fir pitch was nearly as active as Alaska yellow cedar essential oil against *A. bovis* but showed no other activity against bacteria other than *C. perfringens*. As expected, Douglas fir and ponderosa pine methanol extracts showed no antimicrobial activity.

We examined the major components of western juniper for antimicrobial activity in two concentrations that would be expected in the raw extracts (Table 2). The cedrenes showed significant activity against the test organisms except *F. necrophorum*. Cedrol did not show any activity. Nootkatin is the major component in Alaska yellow cedar and it was particularly active against *C. albicans*.

Conclusions

Western juniper showed significant antimicrobial activity against all of the test microorganisms. These results are even more promising is light of the examination of the major components in western juniper. The activity of

the cedrenes against the test microorganisms substantiates the antimicrobial activity shown in Table 1. Western juniper outperformed western red cedar in antibacterial activity but was not particularly effective against the test yeast. Alaska yellow cedar was clearly more potent against all of the test microorganisms but is associated with respiratory and skin irritation. We conclude from our study that western juniper does possess antimicrobial activity and may aid in reducing pathogenic microbes associated with animal foot disease.

Table 1. Minimum inhibitory concentrations for tree wood extracts[1].

	Minimum Inhibitory Concentration[2]			
Species[3]	*Fusobacterium necrophorum*	*Clostridium perfringens*	*Actinomyces bovis*	*Candida albicans*
Juniper Heartwood	1.45	1.55	.017	>2.0
Juniper Heartwood Oil	>2.0	1.1	0.55	1.8
AYC Heartwood Oil	0.15	0.0875	0.0525	0.275
WRC Heartwood Oil	>2.0	>2.0	>2.0	0.2
Douglas Fir Heartwood	>2.0	>2.0	>2.0	>2.0
Douglas Fir Pitch	>2.0	1.5	0.075	1.9
Ponderosa Pine Sapwood	>2.0	>2.0	>2.0	>2.0

[1]MIC is defined as the concentration of extract that allowed no increase in absorbance after 6.5 hr. of incubation.

[2]MIC is expressed in mg/mL. Methanol extracts and essential oils were dissolved in methanol:Tween-80 (4:1 v/v). N=2

[3]Abbreviations: (AYC) Alaskan yellow cedar and (WRC) western red cedar.

Table 2. Antimicrobial effect of pure components of Juniper and Western red cedar[1].

Component Concentration (mg/mL)[2]				

Compound	*Fusobacterium necrophorum*	*Clostridium perfringens*	*Actinomyces bovis*	*Candida albicans*
Nootkatin[3]	>1.5	0.55	>1.5	0.05
α -Cedrene[4]	>1.0	<0.5	<1.0	<0.05
β -Cedrene[4]	>1.0	<0.5	<0.5	<1.0
Cedrol[4]	>1.0	>1.0	>1.0	>1.0

[1]Pure compounds were tested at concentrations reflecting the percentage in the raw extract (n=2).

[2]Compounds were dissolved in methanol:Tween-80 (4:1 v/v).

[3]Nootkatin is the major compound in the heartwood oil of Alaskan yellow cedar.

[4]Major components of western juniper.