Antimicrobial Properties of Heartwood, Bark/Sapwood and Leaves of *Juniperus* Species

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Hexane and methanol extracts of heartwood, bark/sapwood and leaves of twelve taxa of *Juniperus* from the United States were assayed for antifungal and antibacterial activities. The hexane extract of the heartwood of several junipers appeared comparable in antibacterial activity to streptomycin. Antibacterial activity of the hexane extracts from the bark/sapwood of *J. monosperma* and *J. californica* were comparable to streptomycin. No appreciable antibacterial activities were found in the leaf extracts from any species examined. No antifungal activities comparable to amphotericin B were found in either hexane or methanol extracts of the heartwood nor from the bark/sapwood. Antifungal activity against *Cryptococcus neoformans* comparable to amphotericin B was found in the hexane extract of the leaves of *J. occidentalis* var. *australis*. The methanol extracts from the leaves of *J. osteosperma* and *J. californica* had antifungal activities comparable to amphotericin B against *Trichophyton mentagrophytes*.

Keywords*: *Juniperus*; antifungal; antibacterial; *Cryptococcus neoformans*; *Trichophyton mentagrophytes

INTRODUCTION

Juniper wood is the domestic source of cedarwood oil for the United States but the Junipers are also known to contain natural wood preservatives (Guenther, 1952). In fact, the preferred status of juniper wood (cedar) for use as fence posts comes from a long history of its use in wet lands. The control of wood rot and termites is a perennial problem in most parts of the United States and the world. Many of the methods for wood preservation have used arsenic and/or chlorinated hydrocarbons which are environmentally hazardous. Carter (1976) has found that *Reticulitermes flavipes* Kollar (southern termite) could not survive on sawdust from *J. virginiana* nor could they survive on filter paper treated with a pentane extract of the *J. virginiana* sawdust.

Adams (1987) has recently reported on the yields of the heartwood volatile oils from 12 taxa of *Juniperus*, and noted that in addition to the two species currently utilized (*J. ashei* Buch. and *J. virginiana* L.), two additional species of juniper of the United States might be commercially harvested: *J. erythrocarpa* Cory and *J. scopulorum* Sarg. These species were also examined for their potential as sources of phytochemicals (Adams, 1987). Because plant materials were collected for these analyses an opportunity became available to examine the antibacterial and antifungal activities of the heartwood, bark/sapwood and leaves of these juniper taxa. Previous examination of the hexane and methanol soluble extracts of the leaves of *J. monosperma* revealed considerable bioactivity (McChesney and Adams, 1985).

The purposes of this study were to determine the antibacterial and antifungal activities of the heartwood, bark/sapwood and leaves of the principal *Juniperus* species of the United States.

MATERIALS AND METHODS

Samples of wood and herbarium vouchers were collected from *J. ashei* (*Adams 5007–5009*, 9 km W of Ozona, Crockett Co., TX; *Adams 5010–5016*, 2 km E of Junction, Kimble Co., TX); *J. californica* 'A' (*Adams 5067–5071*, 13 km NE of I-40, Granite Mtns., San Bernardino Co., CA) and *J. californica* 'B' (*Adams 5072–5076*, 30 km SE of Yucca, Yuma Co., AZ) ('A' and 'B' refer to the two chemical races discovered by Vasek and Scora (1967) and reconfirmed by Adams, von Rudloff, and Hogge (1983) using leaf volatile oils); *J. erythrocarpa* (*Adams 4987–4996*, 32 km N of Alpine, Jeff Davis Co., TX); *J. deppeana* (*Adams 4974–4983*, 32 km NW of Ft. Davis, Jeff Davis Co., TX); *J. monosperma* (*Adams 5027–5036*, 2 km W of Santa Rosa, Guadalupe Co., NM); *J. occidentalis* (*Adams 5077–5086*, 8 km W of Juntura, Malheur Co., OR); *J. occidentalis* var. *australis* (*Adams 5057–5066*, 2 km W of Sonora Jct., Mono Co., CA); *J. osteosperma* (*Adams 5047–5056*, 25 km E of Monticello, San Juan Co., UT); *J. pinchotii* (*Adams 4997–5001*, 28 km E of Ft.

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Stockton, Pecos Co., TX); (*Adams 5002–5006,* 10 km W of Sheffield, Pecos Co., TX); *J. scopulorum* (*Adams 5037–5046*, 5 km E of Clines Corner, Torrance Co., NM); and *J. virginiana* (*Adams 5017–5025*, 7 km W of Bastrop, Bastrop Co., TX). Voucher specimens are deposited at Baylor University.

The samples consisted of wood (20 cm long × 5–10 cm in diameter) and leaves (400 g). All samples were kept cool (February collections) in the field and then frozen in the lab until analysed.

The wood samples were separated into heartwood and bark/sapwood; each subsample was then kept separate. Portions of the heartwood, bark/sapwood and leaves were dried (48 h, 100 °C) to determine the percent moisture. Extracts were obtained from fresh heartwood, bark/sapwood and leaves by Soxhlet extraction of each set of materials for 6 h (Adams and McChesney, 1983). In each case the first solvent used was hexane and the second (sequential) solvent used was methanol. The material was dried (4 h at 70 °C) after the hexane extraction to remove the hexane before extraction with methanol (see Adams, Balandrin and Martineau, 1984, for detailed notes on the extraction protocol).

Qualitative antimicrobial screening was carried out using the agar-well diffusion assay (Clark *et al.*, 1981) against the following organisms: *Bacillus subtilis* 6633, *Staphylococcus aureus* 6538, *Escherichia coli* 10536, *Pseudomonas aeruginosa* 15442, *Mycobacterium smegmatis* 607, *Cryptococcus neoformans* 32264, *Saccharomyces cerevisiae* 9763, *Pycnoporus sanguineus* 14622, *Aspergillus flavus* 9170, *Aspergillus fumigatus* 26934, *Trichophyton mentagrophytes* 9972.

All test organisms were obtained from the American Type Culture Collection (Rockville, MD USA). Crude extracts and fractions were tested at a concentration of 20 mg/mL in ethanolic or aqueous ethanolic solution. Results of the qualitative screen were recorded as the average radius of the zone of inhibition surrounding the well containing the test solution (after 48 h incubation for bacteria and 72 h incubation for fungi) and are reported according to the following code: − = no activity; ± = questionable activity; + = 1–3 mm zone radius; ++ = 4–7 mm zone radius; +++ = 8–12 mm zone radius; ++++ = ≥13 mm zone radius. Streptomycin sulfate (1 mg/mL) and amphotericin B (1 mg/mL) were included as positive controls for antibacterial and antifungal activity, respectively.

RESULTS AND DISCUSSION

Antibacterial activity was assayed for the heartwood, bark/sapwood and leaf extracts. Essentially no activity was found against *E. coli* from the hexane extracts of the heartwood (Table 1). Nearly all species showed activity against *S. aureus*, particularly in the hexane extracts of the heartwood (Table 1). Little or no activity was observed against *P. aeruginosa* (Table 1). Almost all the species had antibacterial activity against *B. subtilis* and *M. smegmatis* (Table 1). The results of this screen indicate that follow-up research (bio-guided fractionation) will be needed.

Antibacterial activity of the bark/sapwood extracts was very similar to that of the heartwood extracts (cf. Tables 1 and 2). In general, more activity was found in the non-polar extracts than the polar extracts (Table 2) and activity was observed against the Gram-positive bacteria, *S. aureus* and *B. subtilis*, and the acid-fast bacterium *M. smegmatis*. Activities comparable to streptomycin were found in the hexane extract from *J. californica* and *J. monosperma* (Table 2).

The leaf extracts exhibited less antibacterial activity, in general, than the wood extracts (cf. Tables 1, 2 and 3). However, these extracts were from unground leaves and some of the active components may be sequestered in glands. Small antibacterial activities were found in both the non-polar and polar

Table 1. Antibacterial activity of juniper heartwood extracts after 48 hours

Species	*E. coli*		*S. aureus*		*P. aeruginosa*		*B. subtilis*		*M. smegmatis*	
	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	±	−	++	+	−	−	++	NT	+++	+
J. californica 'A'	+	−	+	+	−	−	+	+	++	+
J. californica 'B'	−	−	+	+	−	−	+	NT	+	+
J. deppeana	−	−	++	+	−	−	++	+	+++	++
J. erythrocarpa	−	−	++	+	−	−	NT	NT	+++	−
J. monosperma	+	−	+	+	−	−	+	NT	++	+
J. occidentalis var. *australis*	−	−	++	+	−	−	++	+	++	+
J. occidentalis var. *occidentalis*	+	−	++	+	−	−	++	+	++	+
J. osteosperma	−	−	++	+	−	−	++	+	++	+
J. pinchotii	NT	−	NT	+	NT	−	NT	NT	NT	+
J. scopulorum	−	−	++	++	−	±	++	+	++	++
J. virginiana	−	−	++	+	−	−	++	+	+++	+
Streptomycin sulfate 1 mg/mL	++		+++		++		+++		++++	

HEX, hexane extract. MEOH, methanol extract.
Activities are reported as: + = 1–3 mm; ++ = 4–7 mm; +++ = 8–12 mm; ++++ = greater than 12 mm (average radius of the zone of inhibition). (−) = no inhibition.
Extracts were tested at 20 mg/mL, 100 μL applied. NT, not tested.

Table 2. Antibacterial activity of juniper bark/sapwood extracts after 48 hours

Species	*E. coli*		*S. aureus*		*P. aeruginosa*		*B. subtilis*		*M. smegmatis*	
	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	−	−	++	+	−	−	++	+	−	±
J. californica 'A'	−	−	++	+	−	−	++	+	+	+
J. californica 'B'	−	−	++	+	−	−	+++	+	+	+
J. deppeana	−	−	++	+	−	−	+	+	+	+
J. erythrocarpa	−	−	++	+	−	−	++	+	+	+
J. monosperma	−	−	+++	+	−	−	++	+	+	−
J. occidentalis var. *australis*	−	−	++	+	−	±	++	+	++	+
J. occidentalis var. *occidentalis*	−	−	++	+	−	−	++	+	+	+
J. osteosperma	−	−	++	+	−	−	++	+	+	+
J. pinchotii	−	−	++	+	−	−	++	+	++	+
J. scopulorum	−	−	++	+	±	−	++	−	++	+
J. virginiana	−	−	+	+	−	−	++	+	++	−
Streptomycin sulfate 1 mg/mL	++		+++		++		+++		++++	

Abbreviations and symbols as Table 1.

Table 3. Antibacterial activity of juniper leaf extracts after 48 hours

Species	*E. coli*		*S. aureus*		*P. aeruginosa*		*B. subtilis*		*M. smegmatis*	
	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	−	−	±	−	−	−	+	+	+	+
J. californica 'A'	±	−	−	+	−	−	±	+	−	+
J. californica 'B'	−	−	−	+	−	−	−	+	−	+
J. deppeana	−	+	−	−	±	+	+	++	±	+
J. erythrocarpa	−	−	−	+	−	+	+	+	−	+
J. monosperma	−	+	−	−	−	+	+	++	+	−
J. occidentalis var. *australis*	−	−	−	+	−	−	−	+	++	+
J. occidentalis var. *occidentalis*	−	−	+	+	±	−	NT	+	±	+
J. osteosperma	−	−	+	+	−	±	NT	+	+	++
J. pinchotii	+	+	+	−	−	+	+	++	±	+
J. scopulorum	−	+	−	+	−	−	−	+	±	+
J. virginiana	+	+	−	+	−	±	+	++	+	+
Streptomycin sulfate 1 mg/mL	++		+++		++		+++		++++	

Abbreviations and symbols as Table 1.

leaf extracts (Table 3). A more thorough examination of the leaf extracts from ground material is in progress.

Only minor antifungal activities of the heartwood extracts were observed against any of the fungi (Table 4). However, hexane was removed with heat, so no volatiles were present in the hexane extracts. It should be noted that Oda *et al.* (1977) found high insecticidal activity in the volatile oils of *Juniperus recurva*. A study of the antimicrobial activity of the volatile heartwood oils is in progress. Essentially no antifungal activity was found in the bark/sapwood (Table 5), which is a little surprising because the antibacterial activity of the bark/sapwood extracts roughly paralleled the antibacterial activity of the corresponding heartwood extracts (Tables 1 and 2).

The antifungal activity of the extracts from unground leaves (Table 6) was strong against *C. neoformans* and *T. mentagrophytes* from a number of taxa. *Juniperus osteosperma* and both varieties of *J. occidentalis* were particularly active against *C. neoformans* (hexane extract, Table 6). The hexane extracts of these taxa, which are active against *C. neoformans*, are noticeably ineffective against *T. mentagrophytes*. The methanol (polar) extracts of *J. californica* and *J. osteosperma* showed activity against *T. mentagrophytes* (Table 6). This would seem to imply that a different component(s) is active against these two fungi. Almost no activity was found against the other fungi.

Overall, many positive antifungal activities were found in the heartwood and leaf extracts which will warrant a further examination by bio-guided fractionation.

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Table 4. Antifungal activity of juniper heartwood extracts

Species	*C. neoformans* 32264		*S. cerevisiae* 9763		*P. sanquineus* 14622		*A. flavus* 9170		*A. fumigatus* 26934		*T. mentagrophytes* 9972	
	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	+	−	+	−	+	−	±	−	±	−	++	±
J. californica 'A'	±	±	−	−	−	−	−	−	−	−	±	+
J. californica 'B'	−	−	−	−	−	−	−	−	−	−	±	+
J. deppeana	+	−	+	−	++	−	−	−	±	−	++	−
J. erythrocarpa	++	+	+	++	+	−	+	−	±	−	++	+
J. monosperma	+	−	+	−	+	−	±	−	±	−	+	−
J. occidentalis var. *australis*	+	±	+	±	+	−	±	−	±	−	+	+
J. occidentalis var. *occidentalis*	±	+	±	+	+	−	−	−	±	−	+	±
J. osteosperma	+	±	+	−	−	−	−	−	±	−	+	±
J. pinchotii	NT	++	NT	++	NT	±	NT	−	NT	−	NT	+
J. scopulorum	±	±	+	±	+	±	−	−	−	−	+	+
J. virginiana	+	±	+	−	+	−	−	−	±	−	++	+
Amphotericin B 1 mg/mL	+++		++		NT		++		++		++	

Abbreviations and symbols as Table 1.

Table 5. Antifungal activity of juniper bark/sapwood extracts

Species	*C. neoformans* 32264		*S. cerevisiae* 9763		*P. sanquineus* 14622		*A. fumigatus* 26934		*T. mentagrophytes* 9972	
	Hex	MeOH	Hex	MeoH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	±	−	−	−	−	−	−	−	−	−
J. californica 'A'	−	−	−	−	−	−	−	−	−	−
J. californica 'B'	−	−	−	−	−	−	−	−	−	−
J. deppeana	−	−	−	−	−	−	−	−	−	−
J. erythrocarpa	−	−	−	−	−	−	−	−	−	−
J. monosperma	+	−	−	−	−	−	−	−	−	−
J. occidentalis var. *australis*	−	−	−	−	−	−	−	−	−	−
J. occidentalis var. *occidentalis*	−	−	−	−	−	−	−	−	−	−
J. osteosperma	+	−	±	−	−	−	−	−	−	−
J. pinchotii	−	−	−	−	−	−	−	−	−	−
J. scopulorum	−	−	−	−	−	−	−	−	−	−
J. virginiana	−	−	−	−	−	−	−	−	−	−
Amphotericin B 1 mg/mL	+++		++		NT		++		++	

Abbreviations and symbols as Table 1.

Table 6. Antifungal activity of juniper leaf extracts

Species	*C. neoformans* 32264		*S. cerevisiae* 9763		*P. sanquineus* 14622		*A. flavus* 9170		*A. fumigatus* 26934		*T. mentagrophytes* 9972	
	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH	Hex	MeOH
J. ashei	−	+	−	±	±	±	−	−	−	−	±	±
J. californica 'A'	+	+	−	+	−	±	−	−	−	−	−	+++
J. californica 'B'	−	+	−	±	−	−	−	−	−	−	−	+++
J. deppeana	±	+	−	+	−	±	−	−	−	−	−	+
J. erythrocarpa	−	+	−	+	±	−	−	−	−	−	−	±
J. monosperma	−	+	+	+	−	±	−	−	−	−	±	+++
J. occidentalis var. australis	+++	+	+	+	−	−	−	−	−	−	−	+
J. occidentalis var. *occidentalis*	++	−	±	−	−	−	−	−	−	−	−	+
J. osteosperma	++	+	+	±	+	±	±	−	−	−	±	++
J. pinchotii	−	+	−	+	±	−	−	−	−	−	−	−
J. scopulorum	±	+	±	±	±	−	−	+	−	−	−	++
J. virginiana	+	+	±	+	−	±	−	−	−	−	−	++
Amphotericin B 1 mg/mL	+++		++		NT		++		++		++	

Abbreviations and symbols as Table 1.

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