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Biological screening of selected Pacific Northwest forest plants using the brine shrimp (*Artemia salina*) toxicity bioassay

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Abstract

The brine shrimp (*Artemia salina*) bioassay was used to screen 211 methanol extracts from 128 species of Pacific Northwest plants in search of general cytotoxic activity. Strong toxicity (LC_{50} < 100 µg/ml) was found for 17 extracts from 13 species, with highest activity observed for *Angelica arguta* roots at <10 µg/ml. Notably, four species of cedar trees and one of juniper in the family Cupressaceae dominated this group with LC_{50} for heartwood extracts ranging from 15 to 89 µg/ml. Moderate toxicity (LC_{50} 100–500 µg/ml) was found in 38 extracts from 27 species, while weak toxicity (LC_{50} 500–1000 µg/ml) was detected for 17 extracts in 16 species. There were 139 extracts from 99 species that were non-toxic (LC_{50} > 1000 µg/ml). Our subsequent studies of conifer heartwoods with strong activity confirm the assay's value for identifying new investigational leads for materials with insecticidal and fungicidal activity.

Keywords: Brine shrimp lethality, *Artemia salina*, Methanol extracts, Bioactivity

Background

The forests and rangelands of Washington and Oregon are diverse ecosystems ranging from the temperate rainforests of the Olympic Peninsula in Washington to the semiarid shrub-steppe of southeastern Oregon (Franklin and Dyrness 1988). Across this region, fir, pine and cedar species are basic foundations to industries producing lumber and structural wood products. Native Americans have long used many forest plants for foods, medicines and handmade materials to improve daily life (Gunther 1973; Forlines et al. 1992). There remains an interest in the herbal remedies (Moore 1993), and many of the plants still have potential for development of new, natural sources of medicines and insecticides.

The brine shrimp toxicity bioassay is a simple method of screening crude plant extracts for cytotoxicity (Meyer et al. 1982; McLaughlin et al. 1991) and is an indicator of potential antitumor, insecticidal, and fungicidal activity (Michael et al. 1956; Harwig and Scott 1971; McLaughlin et al. 1998). The mode of action causing toxicity is unknown, but the results typically correlate with more specific bioactivity tests. The brine shrimp bioassay has also been used to guide the isolation of bioactive compounds, testing of water quality, and detection of fungal toxins (Nguta et al. 2011; Arcanjo et al. 2012; Gadir 2012). This method is an attractive pre-screen for such activities as it is relatively simple and inexpensive to test large numbers of crude plant extracts in a relatively short time. Most surveys of this type have been carried out on traditional medicinal plants of various cultures from around the world (Pimentel et al. 2002; Krishnarajua et al. 2005; Rahman et al. 2008; Moshi et al. 2010; Ved et al. 2010; Bussmann et al. 2011; Nguta et al. 2011; Oryema et al. 2011; Arcanjo et al. 2012; Gadir 2012; Nguta et al. 2012; Biradi and Hullatti 2014; Khatun et al. 2014). A few studies have targeted forest and savannah plants (Horgen et al. 2001; Adouom 2009; Rizwana et al. 2010; Soonthornchareonnon et al. 2012; Ravikumar et al. 2014).

In this paper we report survey results for some forest plants from the Pacific Northwest to gain a preliminary understanding of which ones may merit further, more

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specific testing with potential for developing new medicines and pesticides to benefit future generations.

Methods

Plant materials

Plants were collected during their active growing seasons in western Washington, western and central Oregon. Voucher specimens were deposited at the Oregon State University Herbarium.

Preparation of extracts

Plant materials were air-dried, ground and then extracted at room temperature for 48 h with methanol. The methanol was analytical grade and freshly distilled prior to use. Extracts were evaporated under vacuum on a rotary evaporator and the residue briefly freeze dried under high vacuum to remove traces of solvent and water, then stored at −20 °C until tested.

Brine shrimp toxicity bioassay

Bioassays of the crude extracts were carried out as described by Meyer et al. (1982) and McLaughlin et al. (1991) on freshly hatched brine shrimp (*Artemia salina* Leach). Triplicate samples of each extract were tested initially at concentrations of 10, 100 and 1000 ppm (μg/mL) in vials containing 5 mL of brine solution and 10 shrimp. Survivors were counted after 24 h and the median lethal concentration (LC_{50}) with 95 % confidence intervals calculated using Probit Analysis.

Results

Results of the brine shrimp cytotoxicity screening are shown in Table 1. Extracts with LC_{50} values >1000 μg/ml are considered non-toxic (Meyer et al. 1982). Values between 500 and 1000 μg/ml are considered weakly toxic, those between 100 and 500 μg/ml as moderately toxic, and those <100 μg/ml as strongly toxic (Nguta et al. 2012). A total of 211 crude methanol extracts from 128 species, 116 genera, and 49 families are represented. Strong cytotoxic activity was found in 17 extracts from 13 species (Table 2), moderate toxicity in 38 extracts from 27 species, weak activity for 17 extracts in 16 species, and 139 non-toxic extracts from 99 species. The proportions of all extracts by activity category are shown in Fig. 1.

Discussion

There were more than twice as many extracts with moderate activity than there were with strong activity. Moderately active extracts need not be dismissed as unimportant, since Bussmann et al. (2011), Nguta et al. (2012) and others have noted that toxicity can vary significantly due to harvest time, collection location, plant organ or tissue, and solvent used for extraction. Alcohol or organic solvent extracts are often more toxic than aqueous ones, but not always. Extracts from genera and species with the strongest bioactivity can also exhibit a wide range in their levels of activity for the same reasons, thus varying among experiments and research groups. Given this natural variability and our extensive list of genera and species we decided not to attempt cross comparing levels of activity with those observed by others, as it is beyond the scope of this report.

Tissues identified with LC_{50} < 100 μg/ml cytotoxicity have served us as leads for further studies of bioactive extracts and compounds from heartwoods of yellow, incense, and Port-Orford cedars, and western juniper against mosquitoes (*Aedes aegypti*), ticks (*Ixodes scapularis*), fleas (*Xenopsylla cheopis*) or microbes influencing animal and forest health (Johnston et al. 2001; Panella et al. 2005; Dietrich et al. 2006; Manter et al. 2006, 2007; Dolan et al. 2007, 2009). It is worthwhile noting that three of the compounds in yellow or incense cedar heartwoods have different modes of action than other commercially available mosquito adulticides currently in use (McAllister and Adams 2010). New modes of action are particularly relevant in the search for compounds to overcome resistance to existing pesticides.

Conclusion

Natural products from Pacific Northwest forest resources can offer alternative biocides and repellent compounds with activities comparable to synthetic pesticides for control of arthropods of public health concern and forest microbial pathogens. Other bioactive extracts from our

Table 1 Brine shrimp toxicity at 24 h exposure to plant extracts

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Aceraceae				
Acer circinatum	Vine maple	Bark	>1000	
		Leaves	>1000	
Acer macrophyllum	Big leaf maple	Bark	>1000	
		Catkins	>1000	
Adoxaceae				
Sambucus nigra subsp. caerulea	Blue elderberry	Bark	>1000	
		Berries	>1000	
Sambucus racemosa	Red elderberry	Bark	>1000	
Viburnum ellipticum	Oregon viburnum	Leaves	>1000	
Amaranthaceae				
Amaranthus retroflexus	Pigweed	Aerial parts	>1000	
Apocynaceae				
Apocynum androsaemifolium	Spreading dogbane	Aerial parts	88	55–141
Araceae				
Lysichiton americanus	Skunk cabbage	Flowers	>1000	
		Leaves	>1000	
		Roots	>1000	
Araliaceae				
Oplopanax horridum	Devil's club	Berries-green	338	292–573
		Berries-red	239	187–279
		Leaves	>1000	
		Petioles	237	153–372
		Root bark	21	13–32
		Stem bark	35	23–51
Aristolochiaceae				
Asarum caudatum	Wild ginger	Aerial parts	565	364–918
Aquifoliaceae				
Ilex aquifolium	Holly	Leaves	>1000	
Berberidaceae				
Berberis aquifolium	Tall Oregon grape	Berries green	305	245–352
		Berry stems	>1000	
		Flower heads	608	404–4630
Berberis nervosa	Cascade Oregon grape	Leaves	>1000	
		Roots	>1000	
Berberis repens	Low Oregon grape	Leaves	>1000	
Betulaceae				
Alnus rubra	Red alder	Bark	>1000	
		Leaves	>1000	
Corylus cornuta	Hazelnut	Bark	>1000	
Boraginaceae				
Mertensia paniculata	Tall bluebell	Aerial parts	>1000	
Myosotis laxa	Small flowered forget-me-not	Aerial parts	>1000	
Symphytum officinale	Comfrey	Aerial parts	>1000	
Caprifoliaceae				
Lonicera involucrata	Black twin-berry	Leaves	>1000	
		Bark	>1000	
Symphoricarpos albus	Snowberry	Berries	>1000	
		Leaves	>1000	

Table 1 continued

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Chenopodiaceae				
Sarcocornia perennis	Pickleweed	Leaves	>1000	
Compositae (Asteraceae)				
Achillea millefolium	Yarrow	Aerial parts	565	364–918
		Leaves only	300	216–402
		Seeds	>1000	
Ambrosia chamissonis	Silver burweed	Aerial parts	>1000	
Anaphalis margaritacea	Pearly everlasting	Aerial parts	808	403–2800
Antennaria geyeri	Pussy toes (Geyer)	Aerial parts	>1000	
Anthemis cotula	Dog fennel	Aerial parts	246	182–320
		Roots	>1000	
Bellis perennis	Bellis (English daisy)	Aerial parts	454	282–760
Centaurea xmoncktonii	Meadow	Aerial parts	277	203–355
	knapweed	Roots	109	96–152
Centaurea solstitialis	Yellow star-	Aerial parts	>1000	
	thistle	Roots	693	423–1349
Centaurea stoebe subsp. micranthos	Spotted knapweed	Aerial parts	>1000	
		Roots	87	56–135
Chrysothamnus viscidiflorus	Rabbit brush (Green)	Aerial parts	>1000	
Cichorium intybus	Chicory	Aerial parts	>1000	
Cirsium vulgare	Bull thistle	Aerial parts	>1000	
Conyza canadensis	Horseweed	Aerial parts	159	96–267
Ericameria nauseosa	Rabbit brush (Gray)	Aerial parts	579	360–1006
Eriophyllum lanatum	Woolly sunshine	Aerial parts	>1000	
Grindelia integrifolia	Gumweed	Aerial parts	173	107–276
		Roots	99	75–116
Hypochaeris glabra	Cat's ear	Aerial parts	>1000	
Lapsana communis	Nipplewort	Aerial parts	>1000	
Leucanthemum vulgare	Oxeye daisy	Aerial parts	16	10–25
		Roots	164	139–183
Madia sativa	Tarweed	Aerial parts	>1000	
Matricaria discoidea	Pineapple weed	Aerial parts	192	160–208
Senecio jacobaea	Tansy ragwort	Aerial parts	>1000	
Solidago canadensis	Canada goldenrod	Aerial parts	827	458–2214
Sonchus asper	Prickly sow	Leaves	>1000	
	thistle	Roots	>1000	
Symphyotrichum subspicatum	Douglas aster	Aerial parts	>1000	
Tanacetum vulgare	Common tansy	Aerial parts	62	39–93
Tragopogon porrifolius	Salsify	Aerial parts	>1000	
Convolvulaceae				
Convolvulus arvensis	Orchard morning glory	Aerial parts	>1000	
Cornaceae				
Cornus nuttallii	Dogwood	Bark	>1000	
Cupressaceae				
Callitropsis nootkatensis	Yellow-cedar	Foliage	42	27–65
		Heartwood	89	53–114
		Outer Bark	693	423–1349
		Inner Bark	15	8–24
		Sapwood	>1000	

Table 1 continued

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Calocedrus decurrens	Incense cedar	Heartwood	55	35–80
		Sapwood	>1000	
Cedrus deodara[1]	Deodar cedar	Heartwood	15	9–24
		Sapwood	36	30–39
Chamaecyparis lawsoniana	Port Orford cedar	Heartwood	31	23–39
×*Hesperotropsis leylandii*	Leyland cypress	Heartwood	118	81–161
		Sapwood	>1000	
Juniperus occidentalis	Juniper	Berries	>1000	
	(Western)	Leaves	>1000	
		Heartwood	66	56–77
		Inner Bark	>1000	
		Outer Bark	>1000	
		Sapwood	189	116–338
Elaeagnaceae				
Shepherdia canadensis	Soapberry	Berries	387	255–571
		Leaves	>1000	
		Leaves with twigs	>1000	
		Outer Bark	314	174–662
Ericaceae				
Arbutus menziesii	Pacific madrone	Inner Bark	>1000	
		Red berries	>1000	
Arctostaphylos columbiana	Hairy manzanita	Bark	>1000	
		Leaves	>1000	
Arctostaphylos patula	Green leaf manzanita	Aerial parts	>1000	
Arctostaphylos uva-ursi	Kinnikinnick	Berries-red Leaves/stems	>1000	
			>1000	
Chimaphila umbellata	Prince's pine	Aerial parts	155	131–177
		Stems	126	86–170
Gaultheria shallon	Salal	Leaves	>1000	
Rhododendron macrophyllum	Pacific rhododendron	Bark	>1000	
		Leaves	>1000	
Fagaceae				
Quercus garryana	White oak	Galls	>1000	
		Heartwood	301	195–468
		Inner Bark	>1000	
		Leaves	>1000	
Fumariaceae				
Dicentra formosa	Wild bleeding heart	Aerial parts	>1000	
Geraniaceae				
Geranium dissectum	Cut-leaf geranium	Aerial parts	>1000	
Iridaceae				
Iris tenax	Oregon iris	Aerial parts	>1000	
Labiatae				
Prunella vulgaris	Heal all; Self-heal	Aerial parts	>1000	
Stachys cooleyae	Cooley's hedge nettle (False stinging nettle)	Aerial parts	>1000	
Lauraceae				
Umbellularia californica	Oregon myrtle	Heartwood	363	255–488
		Sapwood	>1000	

Table 1 continued

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Leguminosae				
Cytisus scoparius	Scotch broom	Aerial parts	>1000	
Dalea ornata	Prairie clover	Aerial parts	157	95–257
		Roots	313	121–1632
Robinia pseudoacacia	Black locust	Heartwood	>1000	
Trifolium pratense	Red clover	Aerial parts	>1000	
Liliaceae				
Camassia quamash	Camas	Aerial parts	212	150–952
		Flowers	272	148–583
		Leaves	446	256–905
Prosartes smithii	Smith's fairy bell	Aerial parts	>1000	
Malvaceae				
Malva neglecta	Dwarf mallow	Aerial parts	>1000	
Nyctaginaceae				
Abronia latifolia	Yellow sandverbena	Aerial parts	>1000	
Onagraceae				
Chamerion angustifolium	Fireweed	Aerial parts	>1000	
Oxalidaceae				
Oxalis oregana	Oxalis	Aerial parts	281	268–298
Pinaceae				
Abies grandis	Grand-fir	Needles (new)	>1000	
		Needles (old)	>1000	
Picea sitchensis	Sitka spruce	Needles	>1000	
Pinus monticola	Western white	Bark	>1000	
	pine	Needles	504	397–662
Pinus ponderosa	Ponderosa pine	Bark	>1000	
		Heartwood	107	69–166
		Needles	>1000	
		Sapwood	>1000	
Pseudotsuga menziesii	Douglas-fir	Cones-green	>1000	
		Heartwood	663	422–1153
		Needles	>1000	
		Outer bark	>1000	
		Sapwood	>1000	
Tsuga heterophylla	Western	Cones-green	>1000	
	hemlock	Needles	>1000	
		Sapwood	>1000	
Plantaginaceae				
Plantago spp.	Plantain	Aerial parts	>1000	
Polygonaceae				
Rumex spp.	Dock	Roots	923	822–1537
Polypodiaceae				
Polypodium glycyrrhiza	Licorice fern	Roots	>1000	
Polystichum munitum	Sword fern	Leaves	>1000	
		Roots	>1000	
Pteridium aquilinum	Bracken fern	Roots	>1000	
Portulacaceae				
Claytonia sibirica	Siberian miners' lettuce	Aerial parts	>1000	
Primulaceae				
Trientalis latifolia	Western starflower	Aerial parts	539	430–627

Table 1 continued

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Ranunculaceae				
Clematis vitalba	Clematis	Aerial parts	>1000	
Delphinium trolliifolium	Delphinium	Aerial parts	304	190–489
Ranunculus occidentalis	Western buttercup	Aerial parts	>1000	
Ranunculus repens	Creeping buttercup	Aerial parts	>1000	
Rhamnaceae				
Rhamnus purshiana	Cascara	Bark	393	237–698
		Leaves	247	186–667
Rosaceae				
Aruncus dioicus	Goat's beard	Flowers	>1000	
		Leaves	>1000	
		Roots	>1000	
Crataegus douglasii	Black hawthorn	Berries-green	>1000	
		Leaves	>1000	
Holodiscus discolor	Ocean spray	Bark	>1000	
		Flowers	>1000	
		Leaves	>1000	
Malus fusca	Crabapple	Bark	>1000	
Oemleria cerasiformis	Indian-plum	Bark	>1000	
		Stems + leaves + berries	>1000	
Potentilla pacifica	Pacific silverweed	Leaves	632	298–2309
Prunus spp.	Cherry	Leaves	>1000	
		Inner Bark	>1000	
		Outer Bark	490	354–614
Purshia tridentata	Bitter-brush	Leaves	870	533–1857
		Roots	691	545–884
		Seeds	144	101–192
Rosa nutkana	Nootka rose	Leaves	>1000	
		Stems	>1000	
Rubus parviflorus	Thimbleberry	Leaves	>1000	
Rubus spectabilis	Salmonberry	Bark	>1000	
		Leaves	>1000	
Rubus ursinus	Blackberry (trailing)	Aerial parts	>1000	
Sorbus scopulina	Mountain ash	Berries	318	308–328
		Leaves	>1000	
Spiraea douglasii	Spirea	Aerial parts	>1000	
Rubiaceae				
Galium aparine	Cleavers	Aerial parts	>1000	
Salicaceae				
Populus spp.	Cottonwood	Outer Bark	>1000	
Saxifragaceae				
Tellima grandiflora	Fringecup	Aerial parts	>1000	
Scrophulariaceae				
Digitalis purpurea	Foxglove	Aerial parts	>1000	
Verbascum thapsus	Common mullein	Aerial parts	>1000	
		Roots	>1000	
Solanaceae				
Solanum nigrum	Black nightshade	Aerial parts	662	422–1153
Taxaceae				

Table 1 continued

Plant family and species	Common name	Part used	LC_{50} (µg/ml)	95 % CI
Taxus brevifolia	Pacific yew	Heartwood	>1000	
Taxodiaceae				
Sequoiadendron giganteum	Giant sequoia	Needles	713	580–878
		Heartwood	206	166–246
Umbelliferae				
Angelica arguta	Sharptooth angelica	Aerial parts	123	94–371
		Roots	<10	–[2]
Daucus carota	Queen Anne's lace	Aerial parts	>1000	
Foeniculum vulgare	Fennel	Aerial parts	>1000	
Heracleum maximum	Cow parsnip	Roots	249	167–384
		Umbels	404	307–496
Oenanthe sarmentosa	Pacific water parsley	Aerial parts	76	48–117
Urticaceae				
Urtica dioica	Stinging nettle	Aerial parts	>1000	
		Roots	>1000	

[1] Endemic to the Indian subcontinent, collected from a tree farm in Oregon

[2] 10 µg/ml was the lowest concentration tested with mean mortality at 90 %

Table 2 Plant species and tissues with strong, <100 µg/ml LC_{50}, brine shrimp toxicity at 24 h exposure to plant extracts

Species	Part used	LC_{50} (µg/ml)	95 % CI
Apocynum androsaemifolium	Aerial parts	88	55–141
Oplopanax horridum	Root bark	21	13–32
	Stem bark	35	23–51
Centaurea stoebe subsp. micranthos	Roots	87	56–135
Grindelia integrifolia	Roots	99	75–116
Leucanthemum vulgare	Aerial parts	16	10–25
Tanacetum vulgare	Aerial parts	62	39–93
Callitropsis nootkatensis	Foliage	42	27–65
	Heartwood	89	53–114
	Inner bark	15	8–24
Calocedrus decurrens	Heartwood	55	35–80
Cedrus deodara[1]	Heartwood	15	9–24
	Sapwood	36	30–39
Chamaecyparis lawsoniana	Heartwood	31	23–39
Juniperus occidentalis	Heartwood	66	56–77
Angelica arguta	Roots	<10	–[2]
Oenanthe sarmentosa	Aerial parts	76	48–117

[1] Endemic to the Indian subcontinent, collected from a tree farm in Oregon

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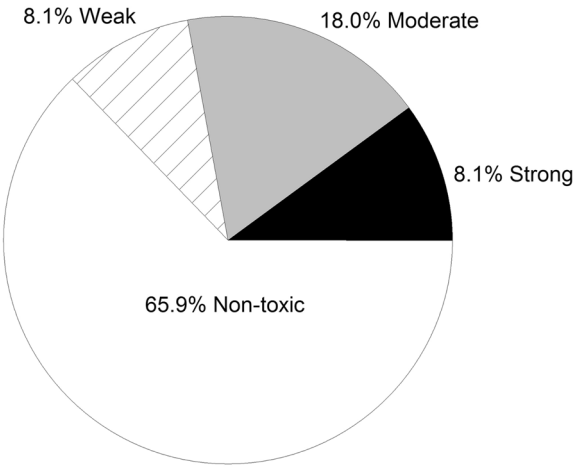

Fig. 1 The percentage of extracts within the four categories of cytotoxic activity

brine shrimp screening need to be investigated further. In addition, other forest plants from this region need to be pre-screened by this method as well to provide a more complete understanding of the potential value for all our forest and rangeland resources.

Authors' contributions

YMK collected plant material, prepared extracts, conducted the bioassays and processed the data. RGK collected some plants, prepared some extracts and co-wrote the manuscript. GC assisted with the bioassays. JJK conceived the study, collected some of the plants, and co-wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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