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 (LC₅₀ < 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 LC₅₀ for heartwood extracts ranging from 15 to 89 µg/ml. Moderate toxicity (LC₅₀ 100–500 µg/ml) was found in 38 extracts from 27 species, while weak toxicity (LC₅₀ 500–1000 µg/ml) was detected for 17 extracts in 16 species. There were 139 extracts from 99 species that were non-toxic (LC₅₀ > 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...
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 (LC50) with 95 % confidence intervals calculated using Probit Analysis.

**Results**
Results of the brine shrimp cytotoxicity screening are shown in Table 1. Extracts with LC50 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 LC50 < 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\textsubscript{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                         |             |
| 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                         |             |
| Symphoricarpus 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-thistle          | Aerial parts | >1000            |                 |
|                           |                              | Roots       | 693               | 423–1349       |
| Centaurea stoebi subsp. micranthus | Spotted knapweed               | Aerial parts | >1000            |                 |
|                           |                              | Roots       | 87                | 56–135         |
| Chrysanthemus viscidiflorus | Rabbit brush (Green)             | Aerial parts | >1000            |                 |
| Cichorium intybus        | Chicory                      | Aerial parts | >1000            |                 |
| Cirsium vulgare          | Bull thistle                 | Aerial parts | >1000            |                 |
| Coreopsis tinctoria      | Horseweed                    | Aerial parts | 159              | 96–267         |
| Ericameria nauseosa      | Rabbit brush (Gray)          | Aerial parts | 579              | 360–1006       |
| Enophyllum 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 jacobae          | Tansy ragwort                | Aerial parts | >1000            |                 |
| Solidago canadensis      | Canada goldenrod             | Aerial parts | 827              | 458–2214       |
| Sonchus asper            | Prickly sow                  | Leaves      | >1000             |                 |
|                           |                              | thistle     | >1000             |                 |
|                           |                              | Roots       | >1000             |                 |
| Symphyotrichum subspicatum | Douglas aster                | Aerial parts | >1000            |                 |
| Tanacetum vulgare        | Common tansy                 | Aerial parts | 62               | 39–93          |
| Tragopogon portulatus    | 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             |                 |
| Plant family and species | Common name          | Part used         | LC₅₀ (µg/ml) | 95 % CI   |
|-------------------------|----------------------|-------------------|--------------|-----------|
| Calocedrus decurrens    | Incense cedar        | Heartwood         | 55           | 35–80     |
|                         |                      | Sapwood           | >1000        |           |
| Cedrus deodara¹         | 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 |           |
|                         |                      |                   |              |           |
| 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        |           |
| Indiceae                | Iris tenax           | 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        |           |
| Plant family and species | Common name                  | Part used  | LC<sub>50</sub> (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      | 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* | Loricice 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      | Fringe-cup                   | 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                 |                              |                  |                   |           |
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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