Podophyllotoxin Content in Leaves of Eastern Red Cedar (*Juniperus virginiana*)

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Abstract

Eastern red cedar (*Juniperus virginiana* L. Cupressaceae) occurs naturally throughout eastern North America and has been identified as a source of podophyllotoxin. Podophyllotoxin is used to manufacture drugs for treatment of cancer, rheumatoid arthritis, genital warts, psoriasis, and multiple sclerosis. Leaf material was collected from *Juniperus* plants located in northern Mississippi once a month from April 2001 to March 2002. The objective of the survey was to examine variations in podophyllotoxin content due to sampling date and plant type. Samples of 100 g of fresh leaf tissue were collected from juvenile, immature, mature male, and mature female plants and then dried, ground, extracted in chloroform, and analyzed with HPLC to measure podophyllotoxin content. Leaf tissue was harvested from the same immature and mature plants each sampling date. Average podophyllotoxin content of juvenile plants was 0.60 mg·g^{-1} compared to approximately 1.45 mg·g^{-1} in immature and mature plant types. Reproductive status, male or female, did not affect podophyllotoxin content. Sampling date significantly affected podophyllotoxin content. Plants harvested in January and April exhibited the greatest podophyllotoxin content (1.56 and 1.45 mg·g^{-1}, respectively) and plants harvested in February and June exhibited the lowest content (1.06 and 1.08 mg·g^{-1}, respectively). Though differences due to sampling date were statistically significant, there appeared to be no obvious pattern or trend. There was no significant interaction between plant type and sampling date. These results indicate that juvenile leaf tissue should be avoided due to its lower podophyllotoxin content compared to that of mature leaf tissue. These results also indicate that podophyllotoxin content of Eastern red cedar can vary as much as 50 % over time, ranging from a low of 1.06 to a high of 1.56 mg·g^{-1}.

INTRODUCTION

Eastern red cedar (*Juniperus virginiana* L. Cupressaceae) has been shown to contain podophyllotoxin, a lignan used in the manufacture of several types of drugs of commercial interest (Stähelin and von Wartburg, 1991; Giri and Narasu, 2000; Lerndal and Svensson, 2000). Drugs are currently prescribed, or are being tested in clinical trials, for treatment of cancer, rheumatoid arthritis, genital warts, psoriasis, and multiple sclerosis (Sabine, 1975; ter Heide et al., 1988; Beutner, 1996; Lerndal and Svensson, 2000; Bedir et al., 2001). Crude plant extracts containing podophyllotoxin have been available as a pharmaceutical compound for hundreds of years, and the compound has a long history of being listed in American and Oriental pharmacopeas.

Podophyllotoxin is currently available from rhizomes and roots of the Indian mayapple, *Podophyllum emodi* Wall. (syn. *P. hexandrum* Royle) Berberidaceae. The plant is native to particular elevations of the Himalayan regions of India, Pakistan, Nepal, and

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China, and is considered endangered due to extensive harvest from the wild (Foster, 1993). American mayapple, *P. peltatum* L. also contains podophyllotoxin, and leaves have been shown to contain high concentrations of the compound (Canel et al., 2001). Since leaves are a renewable resource, American mayapple is being investigated as a potential domestic source of podophyllotoxin (Moraes et al., 2001). However, podophyllotoxin content varies significantly among genotypes harvested from the wild, ranging from 1.1 to 56 $mg \cdot g^{-1}$ on a dry weight basis. Domestication of the higher-yielding genotypes is being investigated (Moraes et al., 2001). This research about Eastern red cedar is part of a larger effort to identify domestic and abundant sources of podophyllotoxin, including research directed toward improving propagation and culture of American mayapple.

Eastern red cedar is distributed throughout 37 states in eastern and central USA and grows in a wide variety of habitats, from swamps to dry ridges (Lee, 1999). The plant readily volunteers in open, undisturbed areas and is often considered a weedy species along fences and roadways. Wood from Eastern red cedar is used in the furniture industry and has been admired for generations for its ability to repel insects that damage clothing. Cedar oil and its derivatives have been shown to disrupt reproductive and developmental cycles in a number of insects (Sabine, 1975). The species is valued for its essential oils, which are used as components in the manufacture of cosmetics, perfumes, soaps, and polishes (Hemmerly, 1970; Pochan, 1977; Lee, 1999). Cultivars with unique horticultural characteristics have been identified and marketed for use in landscaping (Dirr, 1975).

Though Eastern red cedar is known to contain podophyllotoxin, it is not known whether the compound varies in concentration due to influence of environment, time of year, plant age, plant organ, or genotype. Variation of plant constituents is common among medicinal herbs and other plants of commercial interest, as noted with variation of podophyllotoxin content in American mayapple. Taxane, a compound used in the synthesis of drugs for treatment of breast cancer, was found in leaf clippings of *Taxus sp.* in greatest concentrations approximately one month after onset of vegetative growth. As a result, optimum time of harvest varied depending upon geographic location and weather conditions (Elsohly et al., 1997). In contrast, greatest concentration of taxane in bark of European Yew, *T. baccata* L. Taxaceae, was found in October and lowest was found in January (Veselá et al., 1999). Shoots of European Yew collected once a month for one year from two locations exhibited wide variations in taxoid constituents due to genotype and location (Hook et al., 1999). Locality also affected the sabinene and thujone content of *Thuja plicata* J. Donn ex D. Don, Cupressaceae (Buben et al., 1992).

According to Flake et al. (1978), allopatric introgression affects variation and evolution in *J. virginiana* and *J. scopulorum* Sarg. Adams (1986), studied geographic variation in *J. virginiana* and *J. silicicola* (Small) L.H. Bailey based on analysis of volatile leaf terpenoids and leaf morphology. It has been reported that *Thuja* species differ in the amount of essential oils. Based on essential oils content, *T. occidentalis* L., *T. standishii* (Gord.) Carrière, and *T. plicata* were recommended for production by Buben et al. (1992). Significant variation in leaf oil of *J. virginiana* was reported between sexes and between habitats within a single geographic region (Setzer et al., 1992).

Terauchi et al. (1997) used different methods of drying to isolate maximum contents from *Lycium chinensis* Mill. Solanaceae, and reported that freeze-dried leaves harvested in May gave the highest concentrations followed by microwave oven drying. Contents decreased significantly in August. Maximum content of essential oil in subterranean parts of *Valeriana officinalis* L. Valerianaceae, a perennial ornamental plant used in landscape borders, were reported in September whereas valeric acid and its derivatives and valpotriates reached their maximum concentration in February and March (Bos et al., 1998). According to Lohar et al. (1979), newly sprouted leaves contained a very high percentage of sennosides in *Cassia acutifolia* Del. Leguminosae and *C. angustifolia* Vahl., two woody ornamental plants used as landscape shrubs, whereas a decline occurred with the onset of flowering and fruiting. Sennoside contents also decline in the rainy season.

The objective of the survey reported herein was to examine variations of podophyllotoxin content in *J. virginiana* for one year in plants of differing maturities and reproductive status.

MATERIALS AND METHODS

J. virginiana plants sampled in this survey were growing in the wild and located near Tupelo, Mississippi (34.28N, 88.76W; elevation 100 m). Leaves were collected from tips of branches once a month from 15 April 2001 to 15 March 2002. Four plant types were sampled: juvenile, immature, mature male, and mature female. Juvenile plants were defined as being less than 0.5 m in height and sometimes had both juvenile and mature leaves.

Immature plants were defined as being less than 2 m in height, having no juvenile leaves, and not exhibiting male or female flowers. Mature plants, more than 2 m in height, were easily identified as male or female according to flower structure. Mature plants sampled in this study were 10 m or greater in height. Only juvenile leaves were collected on juvenile plants (Fig. 1A) and mature leaves from other plants (Fig. 1B). Each experimental unit consisted of a sample of 100 to 150 g of leaves.

Once a month, one composite sample of juvenile leaves was collected from many juvenile plants ($n \approx 30$), all of which were located in the immediate area of the immature plants. One sample was also collected from each of four immature, two mature male, and two mature female plants. Samples were collected from the same immature and mature plants each month. Samples were placed in a drying oven at 40 ºC for at least 2 days, ground, extracted in chloroform, and analyzed with HPLC to measure podophyllotoxin content against known standards according to Bedir et al. (2001). Two separate extractions were made from each sample and each extraction was analyzed twice by HPLC. Data from extractions and analyses were pooled to obtain a single mean for each sample and then means were evaluated with analysis of variance procedures for unbalanced data and the MIXED procedure of the Statistical Analysis System (SAS Institute, 1999).

RESULTS AND DISCUSSION

Plant type affected podophyllotoxin content of leaves of Eastern red cedar. Juvenile plants had significantly lower podophyllotoxin content than immature, mature male, or mature female plants (Fig. 2). Average podophyllotoxin content of juvenile plants was approximately 40 % of that of immature and mature plant types. Reproductive status, male or female, did not affect podophyllotoxin content. Sampling date significantly affected podophyllotoxin content. Plants harvested in January and April exhibited the greatest content (1.56 and 1.45 $mg \cdot g^{-1}$, respectively) and plants harvested in February and June exhibited the lowest (1.06 and 1.08 $mg \cdot g^{-1}$, respectively) (Fig. 3). Though differences due to sampling date were statistically significant, there appeared to be no obvious pattern or trend due to sampling date (Fig. 3). There was no significant interaction between plant type and sampling date. These data indicate that juvenile leaves should be avoided due to its lower podophyllotoxin content compared to that of mature leaves. These data also indicate that podophyllotoxin content of Eastern red cedar can vary as much as 50 % over time, ranging from a low of 1.06 to a high of 1.56 $mg \cdot g^{-1}$.

Foliage of Eastern red cedar may be available as a waste product of the lumber industry. Since only mature plants are harvested, the foliage could be expected to contain about 1.4 $mg \cdot g^{-1}$ podophyllotoxin (Fig. 2). These contents are low compared to that of American mayapple, which can be as high as 56 $mg \cdot g^{-1}$ podophyllotoxin. Foliage of Eastern red cedar, however, may be more readily available than that of the American mayapple. In addition, foliage of Eastern red cedar appears to contain significantly less α- and β-peltatin, lignans commonly found in mayapple, which can complicate extraction and purification of podophyllotoxin.

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Figures

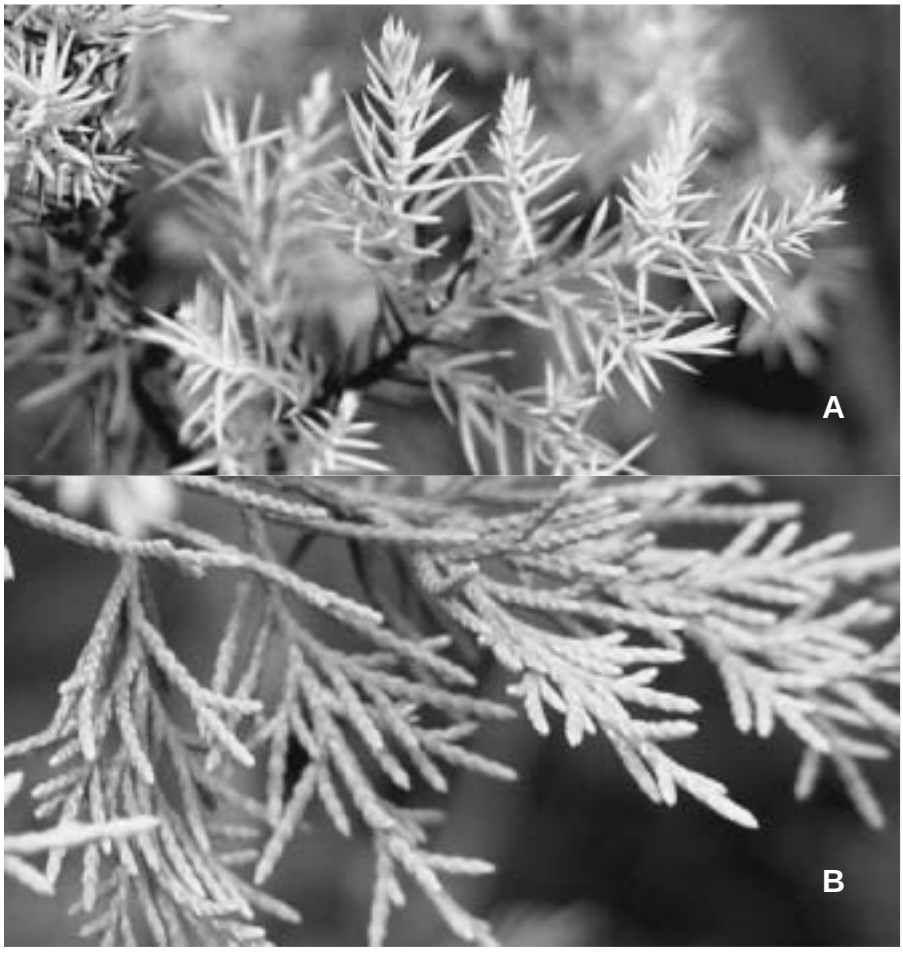

Fig. 1. Type of leaves of Eastern red cedar (*Juniperus virginiana*), (A) Juvenile leaf, (B) Mature leaf.

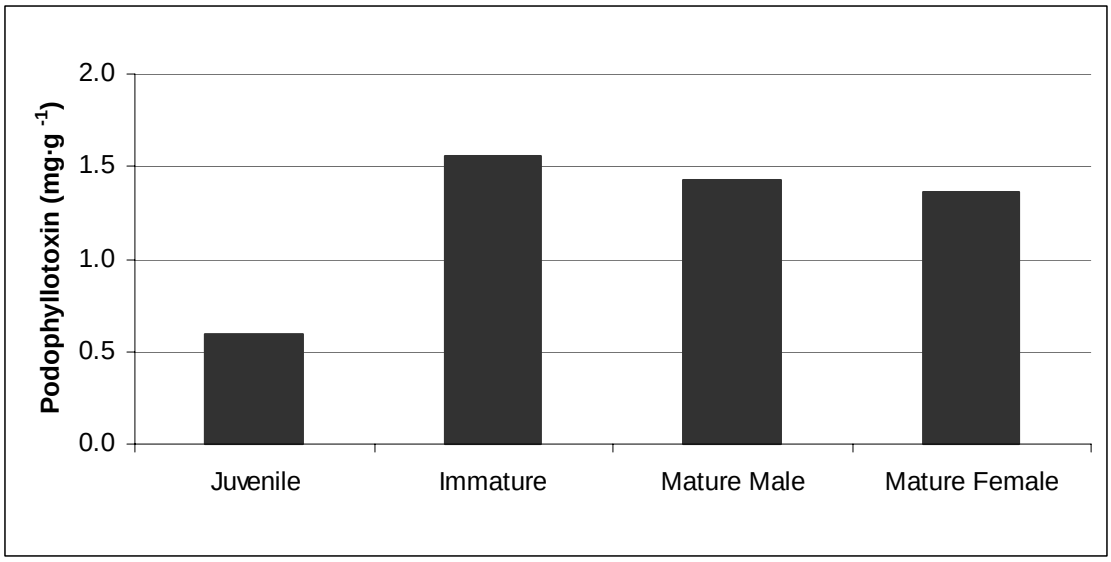

Fig. 2. Podophyllotoxin content of leaves of Eastern red cedar (*Juniperus virginiana*) collected from different plant age groups.

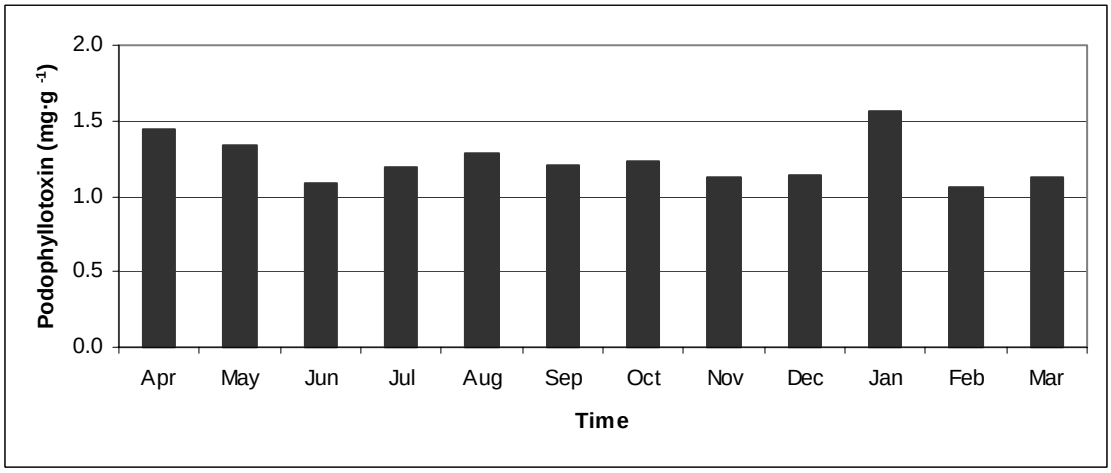

Fig. 3. Podophyllotoxin content of leaves of Eastern red cedar (*Juniperus virginiana*) collected from April 2001 to March 2002.