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SCREENING AND TESTING PHYTOCHEMICALS IN EASTERN REDCEDAR (*JUNIPERUS VIRGINIANA*) FOR DEVELOPMENT OF POTENTIAL ENTREPRENEURIAL OPPORTUNITIES

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Abstract: Eastern redcedar (*Juniperus virginiana*) is often considered a "trash or nuisance" tree. In some states, this species has been declared invasive and management strategies have been adopted to destroy it. However, value-added phytochemical products from eastern redcedar have the potential to create new industries in regions such as Missouri with an abundant redcedar resource. As a first step toward the development of such industries, it is essential to characterize, and quantify the composition of the individual phytochemicals within various redcedar tissues with modern chromatographic, spectroscopic and bioassay technologies, followed by an evaluation of their commercial applications in agricultural, pharmaceutical, and cosmetic industries. In this study, the distilled cedar oil, cedar sawdust and various tissues including roots, leaves, fruits, branches, sapwoods, and heartwood were collected and intensively extracted with solvents. Separation and fractionation of the phytochemicals with a range of polarity were performed by liquid/liquid extractions followed by a reverse-phase liquid chromatography. Bioassays were performed to evaluate the potent biological activities (herbicidal, antifungal, antibacterial, antitermitic, pesticidal, antitumoral activities, etc.) in each fraction. The potent compounds in the extracts showing high bioactivities will be isolated and further purified for chemical characterization and structure elucidation purpose.

INTRODUCTION

A recent study of the Missouri hardwood industry found that much of the industry is over 50 years behind in both its industrial practices and its marketing model. It went on to state that for the majority of businesses that are locked into an outmoded attitude toward hardwood products, there is no enabling mechanism for capturing modern value adding processes as a means for entering new market areas. They concluded that too many small and medium sized producers are generating predominately low value products, even from high grade raw materials, because of their failure to understand how to compete in the higher value market areas (Hackman and Thompson, 2003). The same can be said about many producers in the Missouri red cedar industry.

Eastern redcedar (*Juniperus virginiana*) is often considered a "trash or nuisance" tree. In some states, this species has been declared invasive and management strategies have been adopted to destroy it. However, another more positive side to redcedar has been revealed through a market

research study indicating a profitable expanding marketplace generating $60 million dollars in annual sales. The eastern redcedar market ranges from large firms with gross annual sales over $16 million to small operations with less than $10,000 per year gross annual sales. Critical resources needed to compete in the eastern redcedar marketplace include access to raw material and labor, market knowledge, financial resources, and the cultivation of personal relationships among players in the value chain. (Gold et al., 2005).

Eastern redcedar is an important source of volatile oils. Cedarwood oil has a significant commercial value in a broad range of applications from cold-remedy salves to room sprays and insecticides. Its extensive utilization in a broad range of products is attributable to its unique properties, such as its odor, repellency or toxicity to many pests, antibacterial, antifungal and antitermitic activities. The aromatic oils found in eastern redcedar heartwood repel clothing moths and are widely used in perfumes (Alemayehu et al., 1998; Lawson, 1990). Aromatic oils are toxic to some ant species (Argentine ant and odorous house ant), and eastern redcedar mulch is effective in discouraging ant colonization (Meissner and Silverman, 2001). Eastern redcedar oils are also effective in repelling Formosan subterranean termites (Zhu et al., 2001). Heartwood extractives may inhibit growth of fungi and bacteria (Lee et al., 1999). Eastern redcedar heartwood has approximately 10 times the oil extractives of sapwood. Due to a higher proportion of heartwood to sapwood in closed-canopy stands of eastern redcedar, trees grown under closed stand conditions may contain 4 to 5 times as much oil in the bolewood as open-grown trees of the same diameter (Wittwer et al., 1999).

Value-added oil based products from eastern redcedar have the potential to create new industries in regions such as Missouri with an abundant redcedar resources. As a first step toward the development of such industries it is essential to verify and quantify the composition of the individual phytochemicals within redcedar oil followed by an evaluation of recovery methods, processing and yield (Semen and Hiziroglu, 2005).

MATERIAL AND METHODS

Basically, the distilled cedar oil, cedar sawdust and various tissues including roots, leaves, fruits, branches, sapwood, and heartwood were collected and intensively extracted with solvents. Separation and fractionation of the phytochemicals with a range of polarity were performed by liquid/liquid extractions and reverse-phase liquid chromatography. The isolation and identification of the potential biologically active phytochemicals will be carried out by various modern chromatographic separation techniques, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy. Bioassays were performed to evaluate the potent biological activities (herbicidal, antifungal, antibacterial, antitermitic, pesticidal, antitumoral activities, etc.) in each fraction. The potent compounds in the extract that show high bioactivities were isolated and further purified for structure characterization and elucidation purpose.

RESULTS AND DISSISCUSION

During 2007-2008, several biologically active compounds extracted from ERC leaves have been successfully purified and characterized. The concentrations and chemical structure were confirmed with high performance tandem mass spectrometry (HPLC-MS/MS), gas

chromatography ion-trap tandem mass spectrometry (GC-MS/MS), ^{3}H- nuclear magnetic resonance (NMR), ^{13}C-NMR and 2D-NMR. Many of the active phytochemicals demonstrated strong anti-microbial activities against a wide range of human bacterial pathogens, including *Staphylococcus aureus* and MRSA (Methicillin-resistant *Staphylococcus aureus*), *Bacillus anthracis*, *Bacillus cereus*, *Vibrio* sp., *Listeria monocytogenes*, and *Streptococcus* spp. (Table1). Several isolated compounds have shown a promising potential for various cosmetic and pharmaceutical applications due to its anti-bacterial, anti-inflammatory, anti-fungal, anti-malarial, antioxidant, and anti-tumoral effects. At least one of the identified compounds has been successfully incorporated into the formulation of cosmetic skincare products for acne treatment in the existing market. A recent US patent describing new cosmetic application of this compound for reducing skin inflammation or treating inflammatory disorders, pain or purities was reported by Johnson & Johnson (2005).

	Minimum Inhibitory Concentrations (MIC)	
	($\mu g\ mL^{-1}$)	(μM)
Clostridium sp.	21.4	75
Streptococcus pyogenes (Group A Strep)	85.7	300
Streptococcus agalactiae (Group B Strep)	85.7	300
Listeria monocytogenes	85.7	300
Pseudomonas sp.	85.7	300
Vibrio sp .	142.8	500
Salmonella sp .	No effects	No effects
Staphylococcus aureus (MRSA)	21.4	75
Bacillus anthracis	42.9	150
Bacillus subtilis	85.7	300

Table 1. Minimum inhibitory concentrations of the purified compounds

The results of our bioassay have shown strong activities of the purified compounds not only against melanin development (skin pigmentation), but act against the mouse melanoma cell line B16F10luc, a cell line genetically engineered with green fluorescent protein for bioassay purpose (Figure 1 and Figure 2). The isolated phytochemicals possessed a abitetane diterpene moiety which has shown excellent inhibitory effects on melanin development and tyrosinase activity. They have been recognized by their great potential for skin care application for preventing and ameliorating the pigmentation after sun-burn/spots/freckles/liver spots. (e.g., skin-whitening). This class of diterpenoid has been proven to be highly safe for external skin application.

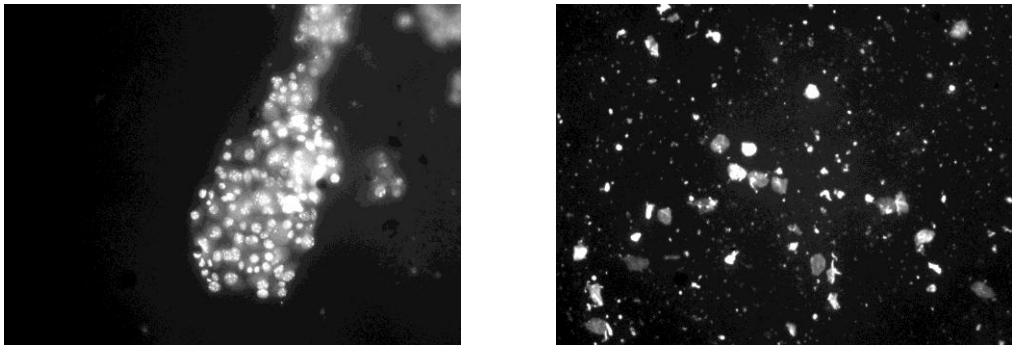

Figure 1. Effects of isolated diterpenoids on survival of mouse melanoma cell line B16F10luc. Melanoma cells in control (left) and cells treated with isolated diterpenoids (right) at concentrations of 200 ppm (0.7μmole/ml). The cell line was genetically engineered with fluorescent protein for bioassay purpose.

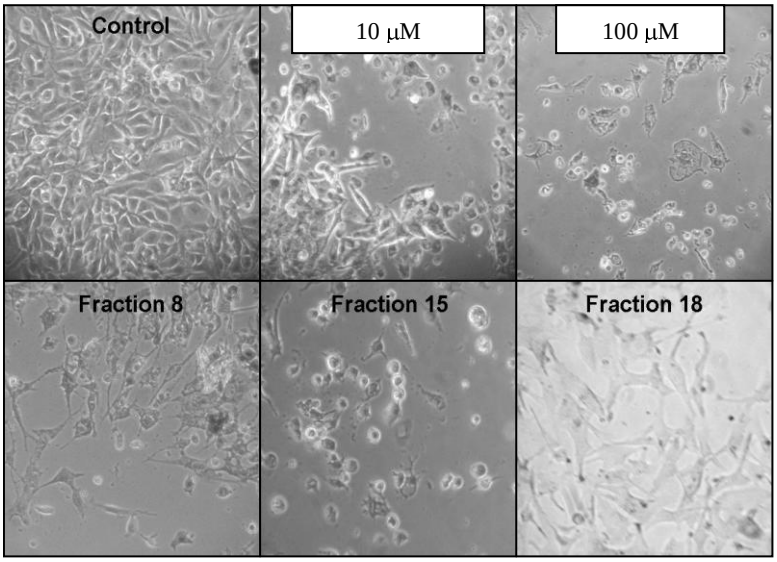

Figure 2. Effects of isolated diterpenoids on survival of the mouse melanoma cell line B16F10luc. Melanoma cells prepared in control and cells treated with purified compound or ECR leaf fractions containing novel isolated diterpenoids at concentrations of 200 ppm (0.7μmole/mL)

Other isolated potent isomeric diterpenoids or abietane diterpene analogs have also demonstrated strong anti-microbial activities against a wide range of human pathogens in our preliminary screening process. However, their applications have not yet been characterized and their potential for commercialization has not been assessed. Figure 3 illustrates the antibacterial (against *Staphylococcus* MRSA and *Bacillus sp.*) and antifungal activities (against yeast) of the isolated fractions containing highly bioactive diterpenoids.

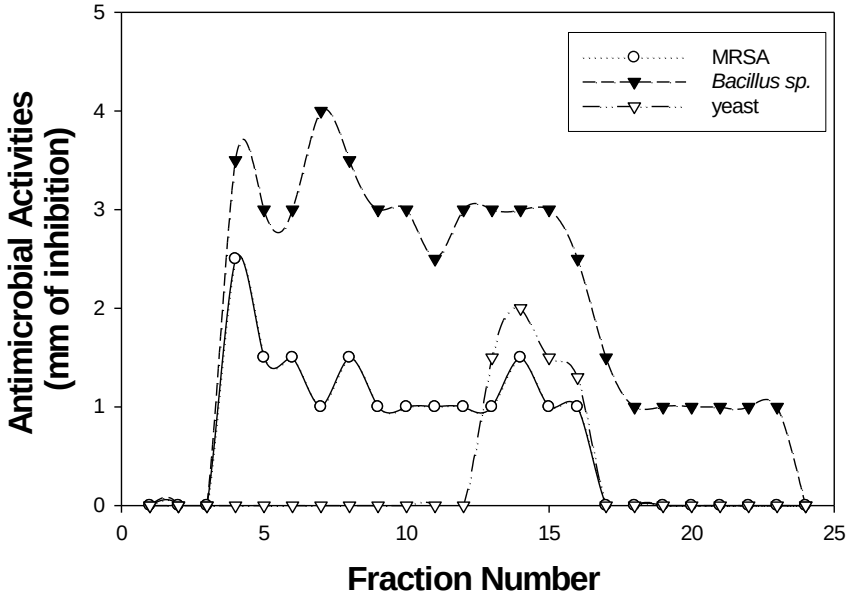

Figure 3. Antimicrobial activities of isolated fractions containing highly bioactive diterpenoids.

CONCLUSIONS

Many classes of isolated biologically active phytochemicals in eastern redcedar have shown promising health benefits. Our current efforts are to continue the screening and isolation processes for potent compounds in the extracts. The compounds in the extracts showing high bioactivities will be isolated and further purified for chemical characterization and structure elucidation purpose.

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