2004

# Study on the termiticidal components of Juniperus virginiana, Chamaecyparis nootkatensis and Chamaecyparis lawsoniana

Yaojian Liu
*Louisiana State University and Agricultural and Mechanical College*

# STUDY ON THE TERMITICIDAL COMPONENTS OF *JUNIPERUS VIRGINIANA*, *CHAMAECYPARIS NOOTKATENSIS* AND *CHAMAECYPARIS LAWSONIANA*

**A Thesis**
**Submitted to the Graduate School of the**
**Louisiana State University and**
**Agriculture and Mechanical College**
**In Partial fulfillment of the**
**Requirements for the degree of**
**Master of Science**
**In**

**The School of Renewable Natural Resources**

**By**
**Yaojian Liu**
**B.S. Shandong Institute of Light Industry, 1994**
**M.S. South China University of Technology, 2001**
**May 2004**

# ACKNOWLEDGEMENTS

The author would like to express his sincere appreciation to several people. I am very grateful for all the guidance and assistance that I received from Dr. Todd Shupe throughout the duration of the project. Dr. Shupe provided very helpful assistance from collection of the seedlings of Eastern red cedar to data analyses, and I will always be grateful.

I would always like to express my gratitude to Dr. Chung Hse for his guidance on experimental design and assistance in experiments. I also appreciate his kind help with my study, life and other aspects.

I would like to express my gratitude to Dr. Niels deHoop for his advice on my experiments and thesis reviewing. Dr. John Jones in the Department of Plant Pathology and Crop Physiology at Louisiana State University (LSU) provided the fungi for our decay tests. Dr. Zhijun Liu in the School of Renewable Natural Resources (School of RNR) and Dr. Charles E. Johnson in the Department of Horticulture at LSU provided the equipment and advice on callus induction and cultivation. Dr. Juan A. Morales-Ramos at the USDA-ARS Southern Regional Research Center in New Orleans and Dr. Ramsay Smith in the School of RNR at LSU helped us with the termite feeding tests. Dr. Hongzhu Song helped me with the induction of the callus.

I also would like to thank my parents. Their persistent support and understanding led me to the achievements I got today.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Termites cause serious damage to wood and wood products. In recent years, increasing attention has been paid to extractives from naturally decay resistant trees. In this study, we compared the termiticidal and antifungal effects of extractives from Alaska yellow cedar (AYC), Port-Orford cedar (POC), and Eastern red cedar (ERC). Pentane and Hexane-Acetone extractives from YC and POC, and pentane extractives from ERC were most favorable in terms of termite protection among the extractives we got. Weight loss of strips treated with 5000 ppm pentane extractives from Eastern red cedar, was significantly lower than those treated with solutions of pentane extractive from the other two species of wood at the same concentration or lower concentrations. Wood blocks treated with pentane extractives from ERC also exhibited significantly lower weight loss than other fractions from ERC and from the other two species at 5000 ppm after 3 weeks exposure to brown rot fungi. Weight loss of blocks treated with hexane-acetone extractives was significantly lower than other treatments after 6 and 9 weeks for the white rot decay test.

Comparing the chemical components in the different tree parts in ERC, some difference were found between the needles, bark and the stem of ERC. Cedrol or widdrol were not detected in the extractives from needles extracted by hexane. From the TLC spectra there were small differences between the components in callus induced from ERC and the seedlings. GC-MS spectrum showed smaller types of components and a bigger difference of the peak areas between components in the callus. A higher concentration of cedrol was detected in the callus than that in the original seedlings. Widdrol was not detected in the extractives from callus or seedlings.

# CHAPTER 1. INTRODUCTION

## 1.1 Termite Control

### 1.1.1 Termites Attack to the Wood Product

Termites are responsible for much of the degradation of wood and other cellulose materials in terrestrial environments. In the United States, concern for wood protection increased after World War II, when the Formosan Subterranean Termite (FST) returned from the orient on boats. The FST has spread across the USA. Protection against FST has been more important since 1998 when the potent organo-chlorine poisons were forbidden.

Termites attack both living trees and wood. They form a big eating machine, degrading wood and other cellulose in the terrestrial environment. In fact, termites can damage many kinds of other materials, such as paper, fabrics, wood structures, and even some non-cellulose as asphalt, asbestos, bitumen, lead, and metal foils (Bultman, 1979).

The Formosan Subterrian Termite (*Coptotermes formosanus* Shirake), a native of Mainland China, has become a more important international structural pest relative to other species of termites. After its initial discovery in the United States in Houston in 1965, it has been distributed to Alabama, California, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Tennessee. *C. fromosnaus* may in some cases displace native *Reticulitermes* spp (Chen, 1998).

It is reported that the annual damage to trees and wooden structures is estimated at $300 million dollars in New Orleans, LA. In 10 years, the termites have destroyed more of metro New Orleans than hurricanes, tornadoes and floods combined. The total global

damage due to the FST is about one billion US dollars (Green, 2000). Therefore there is a great potential market for an effective termiticidal chemical to control the propagation and the damage of termites.

### 1.1.2. Termite Control

There are mainly two types of methods to control termites: chemical and physical barriers. Although they are different types of strategies, they are often combined in the control of termite. This combination of treatment options is typically referred to as Integrated Pest Management.

Physical methods mainly focus on increasing the difficulty for termites to access the wood. For example, Behr et al. (1972) demonstrated an inverse relationship between amount consumed by *R. flavipes* and the specific gravity and hardness of wood; and constructions made of high-density wood can affluence the termite's ability to fragment the wood mechanically. Typical physical methods include proper management of the moisture in wood and thus keeping the wood less susceptible to termites attack.

Chemical methods apply chemical compounds to repel or kill the termite, repel the reproduction or interfere with the communication of the termites. Susceptible wood may be protected by spraying, painting, dipping, or impregnating them with chemicals toxic or repellent to the termites. Since 1970 chromated copper arsenate (CCA) has been the chemical of choice, though there are many kinds of organic compounds or commercial mixtures that are effective for controlling the termite. However, it will no longer be used for residential uses by 2004 due to a voluntary industry support phase is out.

In recent years, natural extractives from wood species with inherent resistance to termites have been widely investigated. These researches are largely driven by two factors: (1) increased concern about the environmental effects of some traditional

preservatives, and (2) that most extractives biosynthesized from wood do not seem to be harmful to the environment. Wood extractives are naturally formed in trees and may be environmentally friendly after application. Developing an extractive-based preservative can help prevent environmental degradation. In fact, the commercial application of plant extractives has a long history, and they are widely used in our everyday life for uses such as medicine, food, cosmetics, and perfume.

## 1.2 Objectives

The ultimate applied objective of this study is to determine an economical method to produce large-scale environmentally acceptable termiticidal preservatives. In the experiments reported herein, we concentrated on four basic objectives.

(1) Determine chemical properties of the different parts of Eastern red cedar (ERC).

(2) Determine chemical differences between callus tissue and seedlings of ERC.

(3) Test the decay effect of the extractives from several decay resistant trees species.

(4) Test the antitermitic effect of the extractives from decay resistant trees.

# CHAPTER 2. LITERATURE REVIEW

## 2.1. Application of Wood Extractives

Humans have been using plant extracts as insecticides since before the time of ancient Romans. People applied the crude mixture called essential oil from natural products in antifungal, antimicrobial, cytostatic, and insecticidal activities nowadays (Franzios, 1997)

There are many plants that have been investigated for termiticidal preservatives, such as vetiver grass oil (Zhu, 2001); seeds (Vanucci, 1992); wood leaves, such as leaves of tarbush (*Flourensia cernua*) (Tellez, 2001); mature leaves of 7 species of Eucalyptus (Lin, 1998); leaves of *Azadirachta excelsa* (Jack) Jacobs (Sajap, 2000); and heartwood from trees. Many species of wood have been extracted, and the efficacy for termite resistance has been tested. These woods include *Taxodium distichum* (L.), (Scheffrahn, 1988); Sawara wood (*Chamaecyparis pisifera* D. Don) (Saeki, 1973); Port-Orford-cedar [*Chamaecyparis lawsoniana* (A. Murr.) Parl.] (McDniel, 1989), bogwood of yakusugi (Yatagai, 1991); *Diospyros virginiana* L. (common persimmon) (Carter, 1978), *Mansonia altissima* and *Piptadeniastrum africanum* (Bultman, 1979); *Juniperus procera* (African pencil cedar) (Kinyanjui, 2000); Taiwania (*T. cryptomerioides*) (Chang, 2001); Melaleuca (*Melaleuca quinquenervia*) heartwood blocks (Carter, 1982); *Lophopetalum wightianum*, *Microcerotermes beesoni* Snyder, *Neotermes bosei* (Snyder) and *Heterotermes indicola* (Wasm.) (Sen-Sarma, 1979); Brazil wood (*Prosopis juliflora*), *Anadenanthera macrocarpa*; *Astronium urundeuva*; *Schinopsis brasiliensis*, *Senna siamea*, *Tabebuia aurea*, *Amburana cearensis*, *Tabebuia impetiginosa* and *Aspidosperma*

*pyrifolium*. (Paes, 2001). Wang (1989) compared the effects of eight species which included (a) *Chamaecyparis obtusa*, (b) *Cunninghamia lanceolata*, (c) *Taiwania cryptomerioides*, (d) *Cryptomeria japonica*, (e) *Acacia confusa*, (f) *Castanopsis carlesii*, (g) red oak (*Quercus sp.*) and (h) Gonystylus bancanus. These plants are broadly distributed from USA, India, Brazil, Africa, and China.

These materials were extracted with organic solvents or hot water to get a crude mixture to test against termites. Most of them were tested in a mixture, and effective compositions were isolated and identified by some of the researchers.

The extracted chemicals may act as feeding deterrent, oviposition deterrents, repellent and toxicant, and trail-following compounds. Many kinds of chemicals are found to be termiticidal due to different resources used. These chemicals include ferruginol and manool (Scheffrahn, 1988), nootkatone (Zhu, 2001; Maistrello, 2001), chamaecynone and isochamaecynone (Saeki, 1973), 2-phenoxyethanol (Laine, 1998), eugenol (Cornerlius, 1997), Terpene (Sharma, 1994), α-terpineol and three sesquiterpene alcohols T-cadinol, torreyol (δ-cadinol), and α-cadinol (McDaniel, 1989), 7-methyljuglone, isodiospyrin (Carter, 1978), cedrol and alpha-cadinol (Chang, 2001), alpha–pinene, p-cymene and 1,8- cineole (Lin, 1998), and aphthalene (Henderson, 1998).

These compositions may have different effects on different species of termites. Most of the experiments were carried out with the FST (Chen, 1996; Valles, 2002). Other species sometimes were also employed, such as *Reticulitermes flavipes* (Green, 2000), viz. *Microcerotermes beesoni*, *Neotermes* [Kalotermes] *bosei* and *Heterotermes indicola* (Sen-Sarma, 1979; Gupta, 1978; Jain, 1990), and white termite (*O. obesus*) (Singh, 2001) collected from sugarcane fields in India. A detailed list of the antitermitic compounds and their origin are listed in Table 1.

Table 1. Termiticidal compounds and their resources.

| Compounds | Sources | Effects | Author |
|---|---|---|---|
| Cedrol and widdrol | Heartwood of *J. virginiana* | Toxic | McDaniel, C.A., 1989 |
| 7-methyljuglone | Heartwood of *Diospyros viginiana* | Toxic | Carter F.L., 1978 |
| Torreyol, α-cadinol T-cadinol α -terpineol | Boards shavings of Port-Orford-cedar, *Chamaecyparis lawsoniana* (A.Murr.) | Toxic | McDaniel C.A., 1989 |
| Chamaecynone | Heartwood of *Chamaecyparis pisifera* D. Don | Toxic | Saeki, I., 1973 |
| Loganin | Heartwood of *Guettarda speciosa* L. | Toxic | Yaga, S.,1985 |
| Carvcrol and citronelled | Hearwood of Hiba Arborvitae (*Thujopsis dolobrata*) | Toxic | Lin, T.S.,1998 |
| Catalponol, catalpalactone | Heartwood of southern catalpa, *catalpa bignonioids* | Toxic | McDaniel C.A, 1992 |
| Eugenol | Plants | Toxic | Cornelius,M.L., 1997 |
| 2-chrysophenol | Derivative of anthraquinone from the heartwood of teak | Toxic | Gupta, B.K, 1978 |
| Nootkatone | Vetiver oil and AYC | Repellent and toxicants | Zhu,B.C.R., 2001 |
| 2-furfuraldehyde | Heartwood of *P. sylvestris* | Repellent | Becker, G., 1971 |
| Ferruginol, manool (more active), nezukol | Heartwood of bald cypress, *Taxodium distichum* | Feeding deterrent | Scheffrahn, R. H., 1988 |
| 2-phenoxyethanol | Ink from Blue ink Papermate ballpoint pens | Trail-following | Chen, J., 1988 |
| 2-bromomethyl-anthraquinone | Derivative of anthraquinone from the heartwood of teak | Attractant and toxic | Gupta, B.K, 1978 |
| Monoterpenoids and sesquiterpenoids | Leaves of tarbush (*Flourensia cernua*) | Not stated | Tellez, M., 2001 |
| Cedrol, α-cadinol | Sapwood or heartwood from *T. cryptomerioides* | Not stated | Chang, S.T., 2001 |

In conclusion, termiticidal extractives from natural resources are very attractive and may have great market potential as future wood preservatives if we can economically produce them on a large scale.

## 2.2. Background of Some Decay and Termite Resistant Trees

### 2.2.1 Alaska Yellow Cedar

Alaska Yellow Cedar (AYC), *Chamaecyparis nootkatensis* (D. Don) Spach, is often used for poles, interior finish, furniture, cabinetwork, novelties, caskets, and hulls of small boats. Some logs have been shipped to Japan to build temples and other important structures, because of its purported resistance to termite attack (Panshin, 1971).

In 1994, Grace compared the effects of wood of AYC, redwood and Douglas-fir and found significantly reduced feeding accompanied by high termite mortality. This result indicated that the heartwood extractives of AYC were toxic to termites (Grace, 1994). When investigation was done on acetone soluble heartwood constituents from AYC, Nootketone was isolated and identified (Erdtman, 1962). In a study on nootketone from vetiver grass oil, nootketone was found to be repellent and toxic to FST (Zhu, 2001). No other possible termiticidal components were reported in the heartwood of AYC.

We think that nootketone may play an important role in determining the termiticidal effects of the extractives from AYC, but we are not sure if only this chemical is responsible for its effect on termite.

### 2.2.2 Eastern Red Cedar

The pentane extractives from Eastern red cedar (ERC), *Juniperus virginiana* L, were found to adversely affect the Native Subterranean Termite, *Reticulitermes flavipes* (Kollar) (Carter, 1974). Most of the termiticidal toxicity is attributable to the sesquiterpene alcohols, cedrol and widdrol, with widdrol appearing to be more toxic than cedrol against *R. flavipes* and *R. virginicus*, but not against *C. formosanus* (McDanial, 1989). Cedrol from ERC was also found to inhibit the oviposition of the coccinellid

beetle (Smith, 1973), and cedrol from Taiwania (*Taiwania cryptomerioides*) has been demonstrated to have the most antifungal effectiveness (Chang, 2003).

### 2.2.3 Port-orford Cedar

Port-orford cedar (POC), *Chamaecyparis lawsoniana* (A. Murr. ) Parl., was found to be the most toxic to Native Subterranean Termite, *Reticulitermes flavipes* (Kollar) in a comparison of the termiticidal effects of 11 North American coniferous woods (Carter, 1974). Termiticidal activity came primarily from α-terpineol and three sesquiterpene alcohols: T- cadinol, torreyol, and α-cadinol. The extractives by hexane and the mixture of hexane, acetone and water had higher contents of α-cadinol and torreyol (McDaniel, 1989). α-cadinol and cedrol were the most effective antifugal components in *Taiwania cryptomerioides* (Chang, 2003).

Although extensive studies have been done with the extractives from Port-orford cedar and Eastern red cedar, no comprehensive comparison on their termiticidal components has been carried out. Also, no reports could be obtained on other termiticidal components in AYC.

This project isolated chemical compounds using extraction methods for all three species. After we obtained the desired parts of the extractives, we performed feeding tests on FST to find out which part of the extractive is the most effective.

## 2.3. Mass Production of the Termiticidal Compounds

From previous studies, we know that secondary metabolites, such as terpenes and sesquiterpenes, play an important role in plants (Obst, 1998). The termiticidal compounds, such as cedrol, widdrol and nootkatone, which are sesquiterpene alcohols and sesquiterpene ketone, are secondary metablites.

There are several approaches for maximizing the biosynthesis of useful secondary metabolites, such as traditional genetic approaches, chemical approaches, cell and tissue culture and transgenetic method. (Ferreira, 1997; Canel, 1999). Induction of callus and cultivation of plant cells are useful methods to reproduce the desired secondary metabolites, and the application of these methods in ERC has not been previously reported. Induction of callus from ERC and the manipulation of the medium to maximize the production of termiticidal components will be meaningful in exploring efficient methods to produce important extractives. Also, further application of suspension cultures of plant cells in a fermentation tank (Zhang, 2001) or inducing root culture from callus (Hu, 1993; Fukui, 1998) will be useful to economically produce the desired secondary compounds in a large scale.

In the previously mentioned studies on the induction of plant cells, various tissues were initiated on many kinds of plant medium, such as needles, stems, roots, embryos and endosperms (Chang, 1996; Wang, 1991), leaves (Ho, 1997; Hu, 1993), and seedling stems (Wang, 1990).

However, the contents of desired compounds may have accumulated in different stages of growth and, therefore, could differ in concentration in different parts of the plant from the same seedling. Studies of the emissions from sapwood and knots showed that knots contain about ten times the amount of volatile terpenes found in sapwood associated with drying of southern pine lumber. Southern pine heartwood contains about five times the amount of volatile terpenes as found in sapwood and more of the volatile compounds present in heartwood were emitted than from knots (Ingram L.L., 2000). Videnšek (1990) examined the taxol content in separated bark, roots, wood, branches, leaves, twigs, and whole seedlings of several species and found that the bark gave the

highest average yield of taxol, and the lowest yield was associated with the wood. Therefore, before the initiation of callus from ERC, it is necessary to know something about the chemical components in the different tree parts.

# CHAPTER 3. MATERIALS AND METHODS

## 3.1. Procedure of the Experiments

The experiments were carried out according to the procedure illustrated in Figure 1. Air-dried lumbers from three species were reduced to powder. Freeze dried callus and seedlings were also reduced to powder. The sample powder was subjected to extraction by various organic solvents and the soluble extractives were finally analyzed with Thin Layer Chromatography (TLC) and Gas Chromatography connected with a Mass Spectrum detector (GC-MS).

## 3.2 Preparation of Raw Materials

### 3.2.1 Materials for the Lumber Extractives

Pieces of AYC, POC, and ERC lumber were air-dried and reduced to powder in a Wiley mill with a 1mm mesh screen, and the 40-60 mesh particles were collected.

Ground samples were sealed in air-tight bags. All the samples were kept in a refrigerator at about 5 $^{o}$C. About 1000 grams of samples were prepared for further extraction. Soon after the extraction, the moisture content of the original powder sample was determined.

### 3.2.2 Materials for the Comparison of the Variation of ERC

Branches of the ERC were collected in April 2002 at Baton Rouge, Louisiana. They were segregated into three parts: (1) green needles, with or without further tips, about 1~1.5 cm long; (2) bark coming from the main branches, and (3) stem wood, which was mainly sapwood. All three portions were freeze vacuum dried to constant weight

and reduced to powder in a small grinder to pass a 10 mesh screen and kept in glass vials in a refrigerator. The fresh and dried weight of the different parts was recorded.

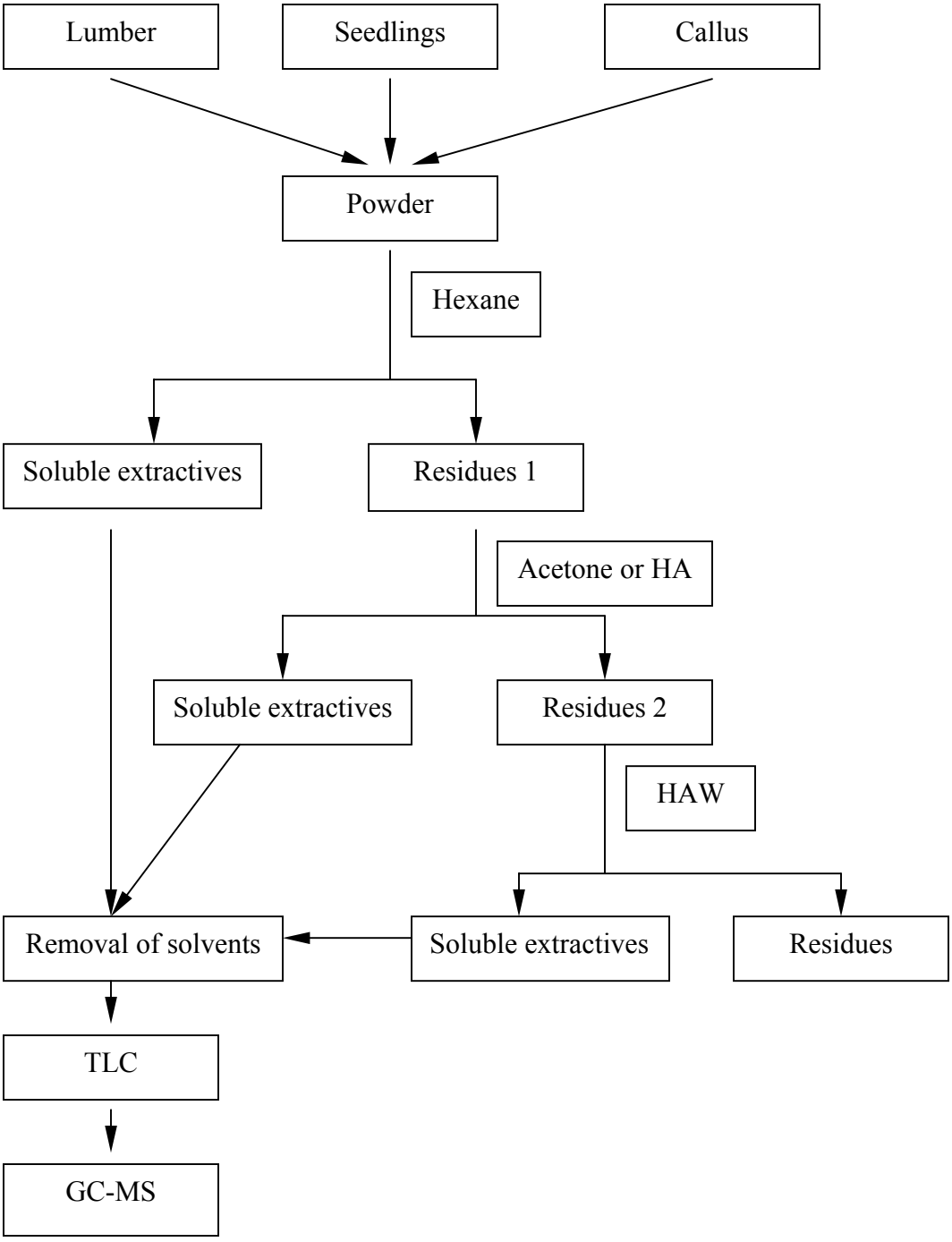



**Figure 1. Procedure for chemical extraction and analysis.**

Note: HA: mixture of Hexane and acetone (1:1), HAW: mixture of Hexane, acetone and water (44:54:2).

### 3.2.3 Seedlings for Induction of the Callus from ERC

The branches were from two-year old seedlings of ERC, which were about 25 cm in height. Branch tips from the current year's growth were cut to explants about 1.5 cm long. After they were washed for 5 minutes with distilled water, they were immersed in 70% ethanol for 20 seconds for sterilization. Thereafter, the explants were washed with sterilized distilled water and impregnated in 1% bleach solution with two drops of Tween for 3 hours and thereafter in 10% bleach with 2 drops of Tween for 20 minutes. Then, the explants were washed with sterilized distilled water 3 times for 5 minutes each time.

## 3.3 Extraction

About 150 grams of lumber powder was weighed and placed into a crucible and put in a soxhlet extractor. The powder was extracted consecutively with n-hexane, mixture of hexane and acetone (v/v=1:1), and a mixture of volume proportion of 54:44:2 acetone, hexane and distilled water (denoted as HAW). The extraction system was heated by an oil bath. The temperature was kept at 80~90°C. The cooling system and heating bath were adjusted so that the siphoning of each solvent occurs about every hour. Every time the sample powder was extracted for 36 hours. At least three batches of samples were tested to get the average amount of extractives.

During the extraction of different parts of ERC and of the powder of the seedlings and callus, the extraction temperature was kept at 75±2 °C and the extractions lasted for 24 hours.

## 3.4 Concentrations and Removal of Solvent

The solvents were removed with a rotary evaporator at reduced pressure to get the dry extractives. The temperature of the water bath for removing the hexane and acetone

was set at 40°C. To remove the water mixed in the solvents in the HAW extracted fraction, we increased the temperature to 60-70°C.

After every fraction was concentrated, we weighed the total weight of every sample. The extractive content was calculated as the following.

$$\text{Extractive content (\%)} = (w_1-w_2)/(w_3\times(1-m\%)), \quad [1]$$

in which $w_1$ is the total weight of the extractives and the flask (g),

$w_2$ is the weight of the flask (g),

$w_3$ is the weight of the samples weighed to extraction (g),

m% is the moisture content of the sample.

All crude extractives were stored at 5°C in a refrigerator.

## 3.5 Preliminary Components Detection

The extractives were applied to a commercially precoated silica gel plate to perform TLC (thin layer chromatography) with a flowing solvent of mixture of hexane and acetone (hexane/acetone=92.5/7.5). The aim of this procedure included identifying the number of components of different tree parts, distinguishing the difference between tree parts, and different resources (seedlings or callus).

The gel plate used was SILICA GEL IB-2F except we used SILICA GEL G in the comparison of the extractives from seedlings and callus. Samples were dissolved in methanol, pentane or acetone. The gel was sprayed with iodine vapor (0.5% in chloroform) and dried with an air blower. We detected the components with fluorescence light, long and short wavelength ultraviolet light.

After the spots were visualized and labeled, their retention factors (Rf value) were calculated and compared. The Rf values were calculated according to the following formular:

$$\text{Rf value} = \frac{\text{Distance from the original point to the spot}}{\text{Distance from the original point to the front line}}$$

[2].

## 3.6 GC-MS

Finnigan mat GCQ$^{TM}$ GC-MS system was utilized to analyze the chemical components to get the chromatography and mass spectra. This system was equipped with four data libraries, and we mainly used the Libr [terpene], which is for terpene analysis. The column employed was the J&W Scientific DB-5MS column (30 m × 0.25 mm fused silica capillary column, Film thickness is 0.25μm). The carrier gas was helium at 500 Kpa (70 psi). Hot needle injection of 1 μl to 10 μl sample solution was applied under splitless injection mode. Injection port was kept at 250 °C. The column was heated from the initial temperature 45 °C continually at 5~15 °C per minute to 280 °C and held 10 minutes. Full scans 50-650 were started after 3~6 minutes. The helium flow speed was kept constant at 40 cm/sec.

Identification were made by comparisons of the positive-ion Electron Ionization Mass Spectrum of each component in the extractives with that of known chemicals. The retention time of the peaks in the results were compared with authentic compounds in the library data and those in literature. Relative quantifications were obtained by peak integration and summation.

## 3.7 Preliminary Termite Feeding Tests

Wood strips of sweetgum (*Liquidambar styraciflua L.*) were cut to a dimension of about 2×3×1/8 inches. The strips were cleaned and dried with vacuum drier for 2 hours. The original weight of every strip was recorded.

For the purpose of finding out which of the three fractions contains the most amount of termiticidal components, we did a preliminary termite test and compared the antitermitic effects according to the weight loss of the strips per percent of extractives applied. Extractives were diluted to the concentration at which the concentration of the extractives absorbed in the strips would equal to their original concentration in lumber. We can calculate the amount of extractive we need to weigh for a definite amount of solvent. With the formula

$$mnp/(p+v)=c\ , \qquad [3]$$

where the sweet gum absorbs $m$ grams of solvent per gram, the average weight of wood strip is $n$ grams, and the extractive concentration in the original materials is $c$ gram per gram, we weigh $p$ grams of extractive to dilute in $v$ grams of solvent.

We dried and sealed the strips in air-tight bags and applied them to the no-choice feeding test. We put 45 workers and 5 soldiers of *Coptotermes formosanus* Shirake and the strips in the test containers (diameter=75 mm, height=65 mm) and maintained them at 26.5$^{o}$C and 80% relative humidity. The dishes in which all the termites died were evaluated every three weeks. The termites were fed for six weeks. Finally, we calculated the termite mortality (percent) and weight loss (percent) of the wood strip.

The strips were weighed after the feeding test. The amount of wood the termites had eaten was calculated and the termiticidal effect of each extractive was compared.

The following equation was employed to calculate the termite mortality and weight loss.

$$\text{Weight loss (\%)} = (w_1 - w_2)/w_1 \times 100\%,$$

$$\text{Termite Mortality (\%)} = \frac{\text{Number of Dishes Termites all Died}}{\text{Total Number of Dishes}} \times 100\%$$

,

$$\text{Extractive gain} = (w_4 - w_3)/w_3 \times 100\%, \quad [4]$$

where $w_1$ is the weight of the original dry weight (grams) of the wood strips and $w_2$ is their dry weight (grams) after the test, $w_4$ is freeze dried weight of the strips after the treatment and $w_3$ is the freeze dried weight of the strips before treatment. Extractive gain was used to calculate the weight loss of the strips per percent of extractives and referred to the difference between replicates to expel the outliers during the statistics.

## 3.8 Further Termite Feeding Test

### 3.8.1 Preparation of the Wood Strips

Strips were cut from the sapwood of southern yellow pine (SYP) tree with 4 rings per centimeter. The sapwood was cut into 2 × 1 × 0.25 inches samples with the end grain vertical. We selected those with no defects to cut into two 1 × 1 × 0.25 inches matched strips, one of which was treated with the extractive solutions and the other one was used to measure the moisture content. Both of them were freeze vacuum dried for two days before further treatment and their weights were recorded.

Pentane-soluble extractives from the three species were diluted to three concentrations (5,000 ppm; 2,500 ppm; and 1,000 ppm), and every concentration had 5 replicates. Every extractive was dissolved in pentane and the replicate strips were

impregnated in solution for 30 minutes. The pure pentane treated SYP strips were used as a control sample. After the treatment, the treated strips were placed in an oven at 60 °C for 1 hour to evaporate the pentane. Their weights after oven drying were recorded to estimate the extractive retention in the strips, which was again denoted as weight and was used to predict and explain the difference between replicates.

We prepared 50 ml of every extract with every concentration in pentane. The weight of the strips was recorded after treatment with the same method described as above. Every sample had 10 replications.

### 3.8.2 Termite Test

The jars were filled with 150 grams of sand and 30 grams of water. 1.45 grams of *Coptotermes formosanus* Shirake termites (about 400) were weighed to every jar. After one month's feeding, the strips were oven dried to get $w_1$. The total weight of the strips before testing ($w_2$) was calculated according to initial weight of the sample ($w_3$) and the moisture content (MC) detected from the match sample with the following formula.

$$W_2=w_3/(1+MC/100), \quad [5]$$

And the weight loss of the strips were calculated according to the following formula,

$$\text{Weight Loss } (\%)= (w_2-w_1)\times 100/w_2, \quad [6]$$

The data from the tests were analyzed with SAS® software. Extractive retention amount and moisture content were used to exclude possible outliers. A 90% confidence level was applied to compare the significant difference between groups and species.

## 3.9 Decay Tests

Decay tests were carried out according to AWPA (American Wood-Preservers Association) standard E10-91, 'S*tandard Method of Testing Wood Preservatives by Laboratory Soil-Block Culture*' (AWPA, 2000).

### 3.9.1. Extractives

Three fractions of the extractives obtained from the heartwood of Alaska Yellow Cedar (AYC), Port-orford Cedar (POC), and Eastern Red Cedar (ERC) were employed in these tests.

### 3.9.2 Experimental Design

There is one independent variable in this experiment, which is the extractive from the three species of wood. Three dependent variables, the gain of extractives during the treatments, the moisture content after the decay tests, and the weight loss percentage after the decay tests were analyzed. The weight loss percentage was used to evaluate the antifungal effects of the different fractions from the same species and extractives from different species. Two kinds of fungi were tested and one treatment was applied to the sample wood blocks for each of the fungi. The treatment had 9 levels that were 9 different extractives from 3 species of heartwood. Three durations were tested and 6 replicates were employed.

A repeated and nested design was used as the experiment design. Particular attention was given to the difference of weight loss percentage between the levels of treatment. It was assumed the blocks, the wafer, and the inoculums are normally distributed. The effect model was established to be the following:

$$Y_{ij} = \mu + \tau_i + \delta_{j(i)} + \varepsilon_{ijk}, \qquad [7]$$

Where, $\mu$= overall mean, $\tau_i$ =treatment effects and $\delta_{j(i)}$ = replicate effects, $\varepsilon_{ijk}$ =experiment error. Statistical Analysis System (SAS 2000) was used to statistically analyze the data of three dependent variables.

### 3.9.3. Preparation of Test Blocks

The felled sweetgum (*Liquidambar styraciflua* L.) was cut to 0.5 × 0.5 × 0.5 inches (1.27 × 1.27 × 1.27 cm) blocks and the blocks were dried in a freezer drier until constant weight. The blocks were weighed and recorded. Then, the blocks were soaked in each extractive solution at a concentration of 5,000 ppm for 30 minutes and dried in an air hood. After the blocks were freeze vacuum dried overnight, their weights after treatment were recorded.

The wafers were also prepared from sweet gum, with a dimension of approximately 1 /8 ×1 1/8 × 1 3/8 inches (0.3175 × 2.8575 × 3.4925 cm). These feeder strips were cut with the wood parallel to the long dimensions.

### 3.9.4. Preparation of Soil

The soil was removed from the original bottles and all clumps were broken up. The soil was thereafter mixed, air-dried and sifted by a U. S. No. 6 sieve and stored in a larger container. The water holding capacity (WHC) was 32.08±0.63% and pH value of the sand was 5.04±0.03.

### 3.9.5. Preparing Culture Bottles

Square glass culture bottles, 225 ml (8 oz.) were half filled with 118 ml soil substrate. Ten replicates were used to determine how much water was lost during the sterilization. We weighed each filled bottle and sterile the bottles at 103.4 kPa (121 ºC) for 45 minutes for each of two consecutive days with an American Sterilizer (American

Cyclomatic Control, AMSCO ®). We reweighed each bottle and calculated the weight loss during the sterilization.

Then the total amount of water needed to add to each of the culture bottle was as follows,

$$\text{Water (g)} = 1.3 \times \text{WHC} + \text{weight loss of water during the sterilization,} \quad [8]$$

Adding water to each bottle, the bottles were covered with 16 layers of cheesecloth squares (Graphic Arts, 4 in. × 4 inches).

**3.9.6. Inoculation**

Two 1/4 × 1/4 inches (6.35 × 6.35 mm) disks of agar cut from an actively growing culture of fungus were placed on the middle left and right edge of the wafer. After the wafer was covered with fungus, the samples were inoculated with brown rot fungus *Gloeophyllum trabeum* (ATCC isolate 11539) or white rot fungus *Trametes versicolor* (ATCC isolate 42462) for 3, 6 and 9 weeks respectively to check for the fungus resistance ability of the extractives. The incubation room was kept at 26 ºC and an average relative humidity (RH) of 70%.

**3.9.7. Results Analysis**

The freeze dried blocks were weighed immediately before and after testing and the weight loss was calculated according to the formula shown below,

$$\text{Weight loss (percent)} = 100 \times (T_3 - T_4)/T_3, \quad [9]$$

in which $T_3$ is the weight before test and $T_4$ is the weight after test, in both conditions the weights of the blocks are after freeze vacuum dried.

The moisture content of the blocks after incubation was measured following the formula,

$$\text{Moisture content (\%)} = 100 \times (T_2 - T_4)/T_4, \quad [10]$$

in which the $T_2$ is the wet weight of blocks after decay test, and $T_4$ is dried weight of the blocks after test.

## 3.10 Induction and Cultivation of Callus

### 3.10.1 Sterilization of the Explants

New branches growing in current year of two-year old seedlings of Eastern red cedar, which were about 25 cm high, were washed for 5 minutes with distilled water. About 1.5 cm long explants were cut from the branches and immersed in 70% ethanol for 20 seconds to sterilize the contaminants on the surface. Then the explants were flushed with sterilized distilled water and impregnated in 1% bleach solution with two drops of Tween® 20 for 3 hours and thereafter in 10% bleach with 2 drops of Tween® 20 for 20 minutes. The explants were flushed with sterilized distilled water for 3 times and 5 minutes each time.

### 3.10.2. Cultivation Media

B5 and WPM medium were used in this experiment supplemented with sucrose, gelrite and small amount of auxin (2,4-D). B5 medium contains 3.1 g/L B5, 1.8 g/L gelrite and 30 g/L sucrose. WPM medium contains 2.3 g/L WPM, and 1.5 g/L gelrite. The medium solution was adjusted to pH5.6 before the gelrite was added. After the

solution was heated to be clear, the medium solution was distributed into 30 baby food jars with about 33 ml each. Then the jars containing the medium were autoclaved for 20 minutes at 121 ºC.

After the medium was cooled to room temperature, the explants were inoculated on the medium. The growth chamber was set at 25 ºC with RH at about 70%.

**3.10.3. Effects of Different Media on the Growth Rate of the Explants**

Explants were cut to 38 grams and cultivated in the above two kinds of media with three replicates, in which 8 mg/L 2, 4- D was added. Their weights were recorded after 27 days. The growth of the explants was calculated.

**3.10.4. Effects of Auxin on the Growth Rate of the Explants**

Prepare medium with the concentration of 2,4-D at 0, 4, 8 mg/L. Weight the fresh explants before ($w_1$) and after ($w_2$) the 2 weeks' cultivation. Calculate the growth amount after T days (per day per gram of inoculums) with the Kienin formula, which is

$$P= (w_2-w_1)\ 1000/\ (w_1 \times T). \qquad [11]$$

**3.10.5. Induction of the Callus and Propagation**

Applying the optimum medium and auxin concentration for the growth of the explants, we induced the growth of the callus. The calluses were propagated on the same medium.

**3.10.6. Analysis of the Chemical Components in Callus from ERC**

And after 9 months' growth, the calluses were collected and freeze dried. Then the tissue was ground and extracted with hexane. The original seedlings were dried and kept after 6 months. The branches above the soil were cut and dried and ground, too. The

powder of the seedling was extracted by hexane. The extractives were applied to TLC and GC-MS following the same methods described in section **3.5** and **3.6**.

# CHAPTER 4. RESULTS AND DISCUSSION

## 4.1 Comparison of the Three Species

### 4.1.1. Extractive Content in the Three Species

The lumber powder of three species of termite resistant trees (AYC, POC and ERC) was extracted in glassware at room temperature. The solvents were pentane, mixture of hexane and acetone (1:1) (HA) and mixture of hexane, acetone and water (44:54:2) (HAW). After the solvent was evaporated to incipient dryness, the weight of the extractives was recorded in Table 2. About 500 grams of sample powder were extracted. The concentrations of the extractives were based on the dry weight of the powder. The extractive content is listed in table 2.

Table 2. Extractives content in the three species of trees.

| Species and Solvents | | Weight of Extractives (g) | Weight of Powder (g) | Moisture Content in Powder (%) | Concentration of Extractives (%) * |
|---|---|---|---|---|---|
| AYC | Pentane | 4.1235 | 500.8 | 9.122 | 0.906 |
| | HA | 9.9174 | | | 2.1791 |
| | HAW | 10.8687 | | | 2.3881 |
| POC | Pentane | 12.1308 | 500.4 | 10.3647 | 2.7045 |
| | HA | 17.4012 | | | 3.8796 |
| | HAW | 2.3303 | | | 0.5195 |
| RC | Pentane | 11.1040 | 500.5 | 12.7022 | 2.5414 |
| | HA | 4.9198 | | | 1.1260 |
| | Haw | 5.5026 | | | 1.2594 |

*: Extractive concentration was calculated on the basis of O.D weight of the lumber powder.

From Alaska yellow cedar, we got 0.906% of light yellow oil like extractives (denoted by AP) by pentane, 2.18% and 2.39% of dark yellow oil like extractives (denoted by AH) by the mixture of HA and HAW (the extractive is denoted by AW), respectively. From Port-Orford cedar, we got 2.7% of medium yellow oil (PP) by

pentane and 3.88% of yellow oil (PH) by HA, and only 0.52% of very dark yellow extractive (PW) by HAW, which seems more dry and has a shining surface. The extract by pentane from Eastern red cedar is light yellow (RP), which was the most abundant (2.54%); while those from HA (RH) and HAW (RW) were dark red, 1.13% and 1.26%, respectively. The pictures in Figure 2 show the appearance of these extractives.

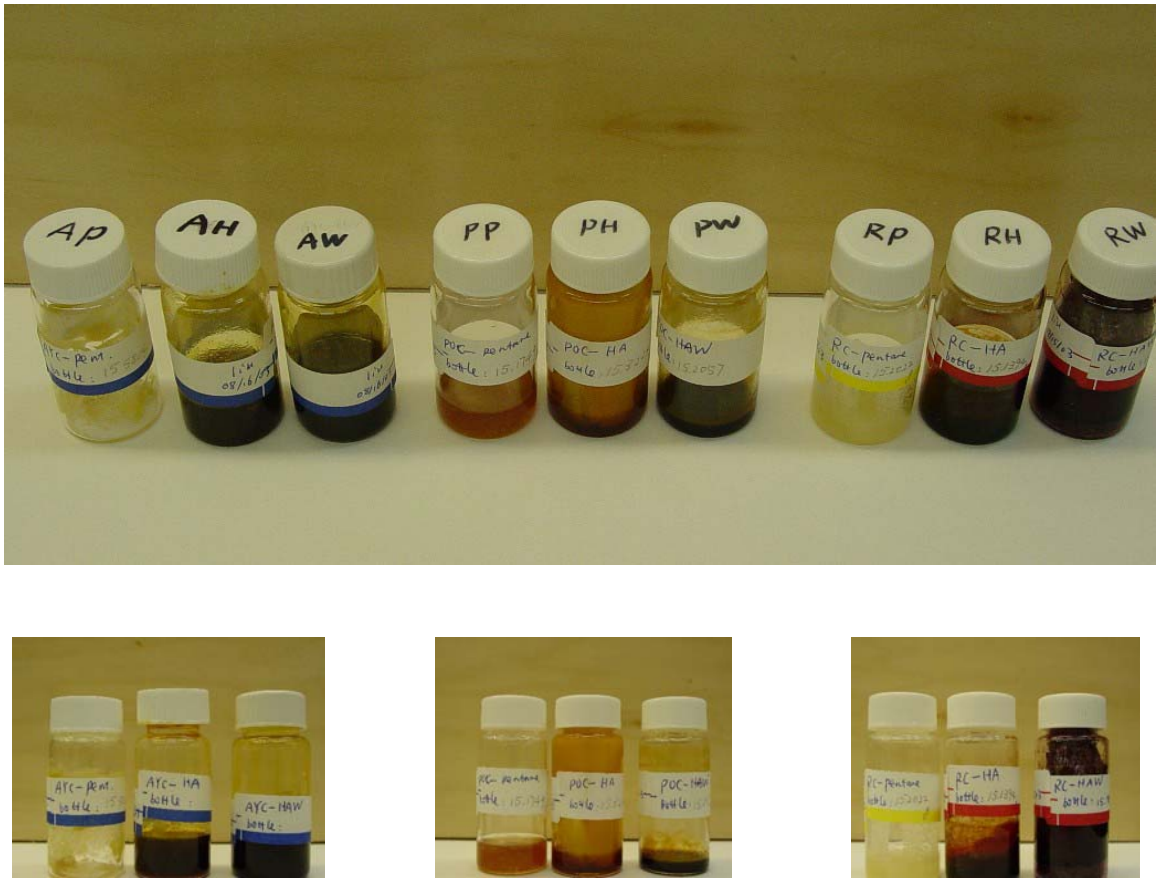



**Figure 2. Extractives from AYC, POC and ERC.**

The low polarity extractives we got by pentane from POC was 2.7%, which was lower than the amount of extractives contained in the bark of POC extracted by hexane (Browning, 1963). This may be a result of the different location of the original material or due to different solvents applied.

### 4.1.2. TLC Results

Every extractive was dissolved in methanol and applied to IB2-F silica gel plate. The extractive solutions and results of thin layer chromatography are shown below.

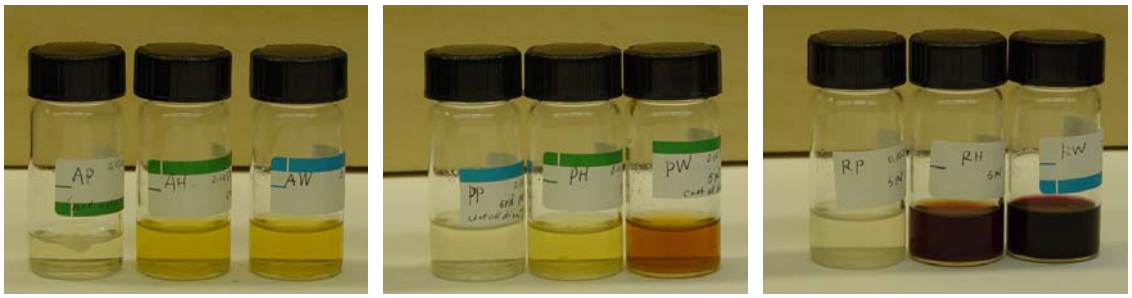



**Figure 3. Methanol solutions of the extractives from AYC, POC, and ERC.**

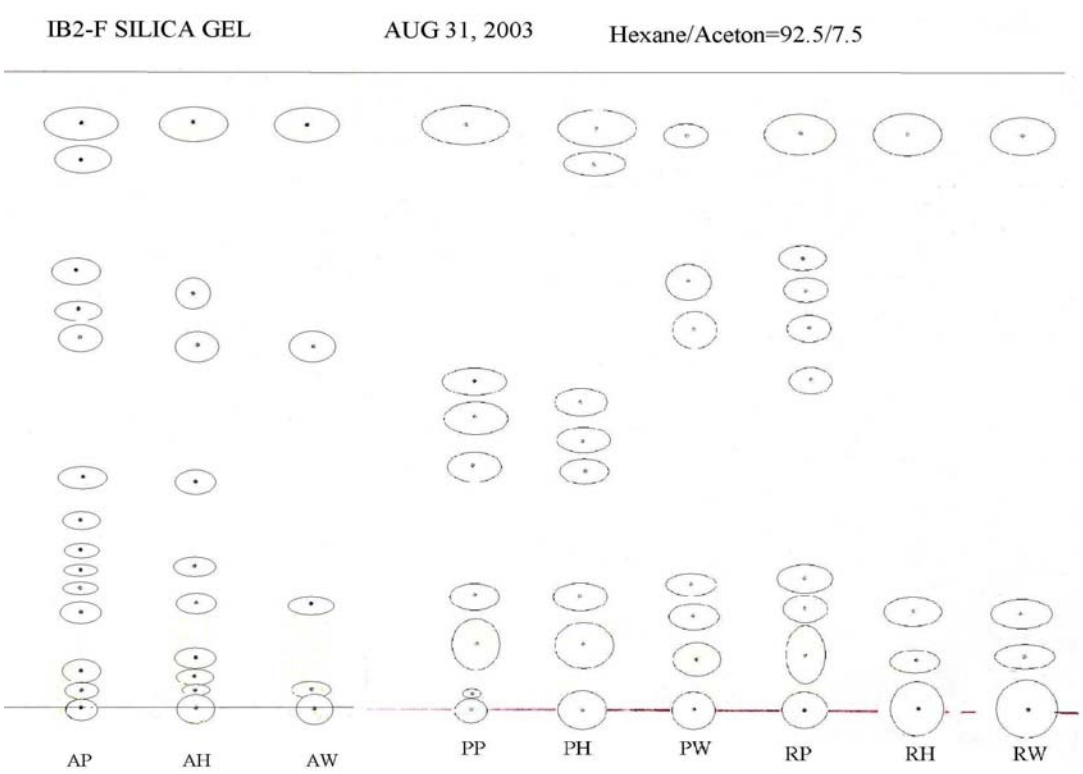



**Figure 4 TLC results of the extractives from AYC, POC, and ERC.**

From Alaska yellow cedar, 14 spots were detected in the pentane extractive, 10 spots were detected in the Hexane-acetone extractive, and only 5 spots were detected in the HAW extractive. From the picture, components in the AH and AW were possibly

contained in AP, though their concentration and proportions may be different from each other. From Port-Orford cedar, 8 spots were detected in pentane extractives (PP), 8 spots were detected in the hexane-acetone extractive (PH), and 7 spots were detected in the HAW extractive (PW). At least 2 spots in PW were not detected in PP or PH and there existed at least 1 spot not detected in the other extractives in the pentane extractive and hexane-acetone extractive. From Eastern red cedar, 9 spots were detected in the pentane extractive (RP), 4 spots were detected in the extractives by hexane-acetone (RH) and HAW (RW), respectively. The components in RH and RW were similar, and it seems they were all included in RP. The pentane extractive contains 5 more spots that were not detected in the other two fractions.

**4.1.3. Preliminary Termite Test Results**

We compared the termiticidal effect of different extractives from the three species with a preliminary termite test. In this experiment we prepared the solutions of different extractives at concentrations approximately proportional to the extractive contents. The objective of this test was to find out which fraction contains the most amounts of the termiticidal components. The control samples (BB) were untreated strips from sweetgum.

In this experiment, pentane was applied to the dissolved fractions extracted by pentane, and hexane-acetone (1:1) mixture was applied to dissolve the rest of the extractives. The first letter in the sample names in Table 2 denotes the original tree name, in which A is Alaska Yellow Cedar, P is Port-orford Cedar and R is Eastern Red Cedar. The second letter denotes the solvent used to get the extractives, in which P is pentane, H is Hexane-acetone (1:1) and W is Hexane-acetone-water.

Table 3. Treatment of sweetgum strips and consumption in the termite test.

| Extractive | Solution Concentr. (%) | Extractive Gain (%) | STD of gain | Weight Loss (%) | STD of Loss |
|---|---|---|---|---|---|
| AP | 2.499 | 2.0043 | 0.1228 | 12.9155 | 4.4886 |
| AH | 5.02 | 6.3992 | 0.3744 | 8.9296 | 2.2553 |
| AW | 6.03 | 5.6007 | 0.8251 | 20.3511 | 9.5304 |
| | | | | | |
| PP | 6.5077 | 4.5008 | 0.6722 | 13.0008 | 3.9933 |
| PH | 8.417 | 8.4753 | 0.7476 | 9.6985 | 1.145 |
| PW | 1.5 | 5.0646 | 0.3258 | 86.0125 | 14.4055 |
| | | | | | |
| RP | 6.009 | 5.55 | 0.4294 | 8.2178 | 3.4527 |
| RH | 2.51 | 4.9319 | 0.5522 | 36.8021 | 5.8396 |
| RW | 3.0728 | 4.6936 | 0.603 | 30.4426 | 10.49 |
| | | | | | |
| BB | | | | 59.611 | 11.423 |

Note: AP: strips treated with Pentane extractives from AYC. AH: Strips treated with HA extractives from AYC. PP, PH and PW denote the pentane, HA and HAW extractives from Port-orford cedar, respectively. RP, RH and RW denote the pentane, HA and HAW extractives from Eastern Red cedar, respectively.

From the results in Table 3 and Table 4, we can draw the following observations. In the extractives from Alaska yellow cedar, all three fractions have some effects on the termites' feeding of the sweetgum. Though the weight loss of the strips falls when AH (8.993±2.26%) was applied comparing to AP (12.92 ±4.49%), the weight loss was not significantly different. However, the concentration of the extractive solution increased to over two fold (2% to 6.4%). It seems the pentane extractives contain the most effective components for controlling the feeding of the termite. Also the hexane-acetone extractive and HAW extractive had some effects on the termites. So, it seems pentane is not a very selective and effective solvent for the extraction of termiticidal components in the AYC. In Table 4, we can see that both of the extractives by pentane and HA caused a higher death percentage of termites after 30 days. This indicates that pentane and HA extracted most of the active components, and the components are more likely toxic to termites than a feeding deterrent.

Table 4. Termite mortality after 30 and 60 days.

| Treatments | Mortality after 30 days (%) | Mortality after 60 days (%) |
|---|---|---|
| AP | 42.9% | 71.4% |
| AH | 57.1% | 85.7% |
| AW | 14.3% | 14.3% |
| PP | 28.6% | 85.7% |
| PH | 28.6% | 71.4% |
| PW | 14.3% | 57.1% |
| RP | 28.6% | 85.7% |
| RH | 14.3% | 71.4% |
| RW | 14.3% | 85.7% |
| BB | 0 | 57.1% |

Note: 14.3% of the samples of BB and PW consumed all the wood by 60 days.

In the extractives from Port-orford cedar, similar results were gotten as for AYC. All three fractions-treated strips had termite resistant effects. Similar with the result of AYC, the pentane extractive from POC had a relative equal effect (13.00±3.99%) to inhibit termite feeding at a lower concentration than the HA extractive (9.70±1.15%). With approximately the same gain of extractives in the strips, the HAW treated strips have less termite resistant ability (25.58±12.93%) than the pentane extractive and HA extractive treated samples; although the difference was not significant. So the pentane extractives or HA extractives probably contain the most effective components in POC. From Table 4, we can see that the effects of the pentane extractive and the HA extractive were higher than that of the extractives from HAW. And it is possible that HAW extractive was less effective than that from AYC in shorter period (30 days) and a little more effective in longer period (60 days).

Among the extractives from Eastern red cedar, the pentane extractive has the most resistant ability (8.22±3.45%) when approximately the same amount of extractives is absorbed. Extractives by HA and HAW had less effect on termite feeding than the

pentane extract, which has a weight loss of 36.80±5.84% and 30.44±10.49, respectively. The termite mortality caused by ERC seems more similar with that of POC. Pentane extracted most of the termiticidal components in ERC, however, there was still some extracted by HA and HAW. And the effect of these components is likely to be a feed deterrent because they caused less mortality in the first 30 days and much higher after 60 days. This can be explained as the components repelled the termites or made the strips more distasteful to them and most of the termites died of starvation.

All the fractions from the three species had some effect on resisting the feeding of termites as compared to the control. But because the control samples are not treated with any solvent, the termiticidal effects should be considered further. While between the three fractions from the same species, it seems the pentane extractive contained most of the active anti-termitic components. However, pentane seems not very selective to the effective component in these three species in extracting the termiticidal compounds. The active components in the three species may lie in a different category.

**4.1.4 Results of Further Feeding Test**

For further comparison of the termiticidal effects of the extractives from the three species, we applied the pentane extractives to a four weeks' feeding test at three concentrations, which is 5000 ppm (H), 2500 ppm (M) and 1000 ppm (L).

The effects of extractives on termite feeding are shown in Table 5. From these results, we found only the extractives from ERC had significant effects (p-value is 0.0026 at 90% confidence level) on the termites at the concentration of 5000 ppm, which lost 36.46±5.292% of its original weight. The termites ate all other strips treated with the extractives and there was no significant difference to the control sample. Since the extractives employed were crude extractives from the heartwood, these results indicated

that the total termiticidal effects of the components in the extractives. The hexane extractive from ERC had the best effect on inhibition of the termites' feeding, so it is possible that the termiticidal components in this extractive is more active or the extractive contained higher concentration of the termiticidal components. It is also possible that the extractives don't contain toxic compounds to termites. Maybe they just make the food distasteful to the termites, so the feeding weight decreases when the strips were treated with extractive from ERC. Extractives from the other two species don't significantly decrease the consumption of feeding strips, which indicates they may not be effective when applied at these concentrations with this method.

Table 5. Feeding test of extractives on termite.

| Groups | Species | Dilution solvent | Concentration (ppm) | Weight loss (%) | Stdev (%) |
|---|---|---|---|---|---|
| AH | AYC | Pentane | 5,000 | 53.65 | 5.28318 |
| AM | AYC | Pentane | 2,500 | 49.02 | 4.0964 |
| AL | AYC | Pentane | 1,000 | 48.23 | 4.5668 |
| PH | POC | Pentane | 5,000 | 49.56 | 4.0964 |
| PM | POC | Pentane | 2,500 | 55.4 | 4.0964 |
| PL | POC | Pentane | 1,000 | 52.61 | 4.5668 |
| RH | ERC | Pentane | 5,000 | 36.46 | 5.292 |
| RM | ERC | Pentane | 2,500 | 52.98 | 4.5668 |
| RL | ERC | Pentane | 1,000 | 50 | 4.0964 |
| BB | | Pentane | | 50.46 | 6.468 |

* All extractives were extracted by pentane. BB is the control sample.

### 4.1.5 Decay Tests

The extractives from the three experimental species of wood were applied for decay testing with brown rot fungus *Gloeophyllum trabeum* (ATCC isolate 11539). The weight loss percentage is shown in Figure 5. The error bar in the figure is the standard error. Six replicates were tested.

From the results, pentane extractives from ERC resisted the growth of brown rot fungus significantly during the first three weeks after the blocks were treated with acetone solution at the concentration of 5000 ppm; the weight loss was 13.3%. The extractives from AYC showed the least resistance (28.25%) to the fungus, which led to 28.25% of weight loss during this period. The weight losses of other extractives were not significantly different (confidence level=90%).

However, the hexane-acetone extractives from ERC exhibited the most significant resistance to the growth of fungi during the 6 weeks' test. The treated blocks lost 26.35% of their original weight. And again the pentane extractives from AYC showed the least resistance to the fungus growth, which caused the treated blocks to loss 39.67% of their original weight. The tests results after 9 weeks indicated no significant difference between the extractives.

These results indicate that extractives from the ERC may inhibit the growth of brown rot fungi for about 6 weeks. With the time elongated, their antifungus effects may decrease. One of the reasons for these results may lay in the treatment of the blocks. Since we only impregnated the blocks in the extractives solution at 5000 ppm for about 30 minutes, it is possible the extractives only stayed on the surface of the blocks or the concentration may be too low for better resistance.

Figure 6 shows weight loss of the blocks exposed to white rot fungi (*Trametes versicolor* (ATCC isolate 42462)). The extractives from Port-Orford cedar by hexane-acetone had some antifungal effects at the concentration of 5000 ppm. After six weeks, the weight losses of the blocks treated by pentane extractives and hexane-acetone extractives from POC were significantly lower than those treated with pentane extractives from AYC and ERC, and there was no significant difference between samples treated

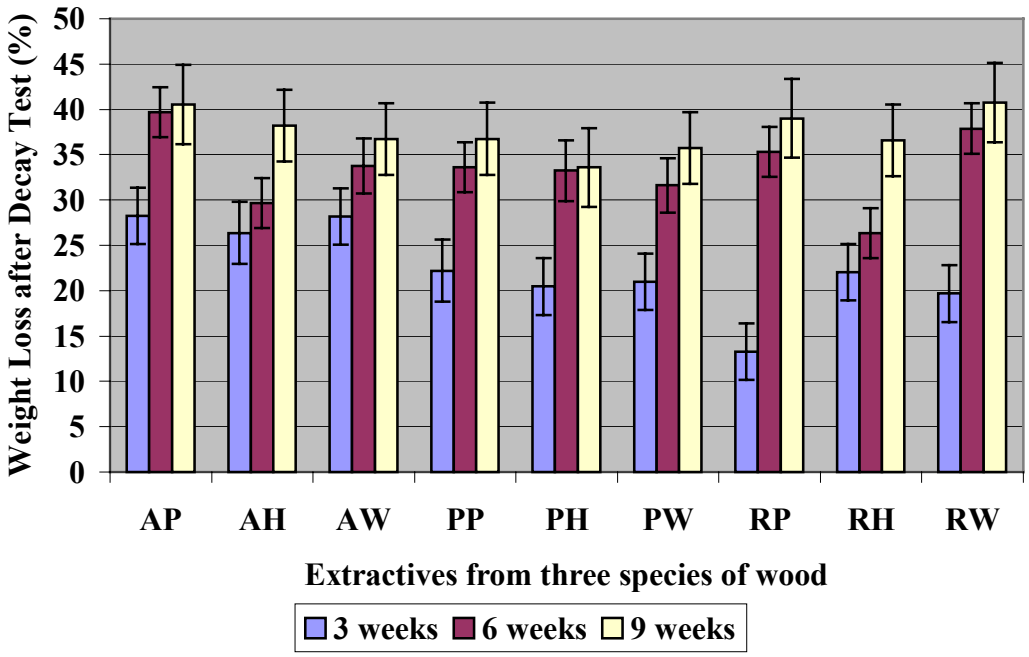



**Figure 5. Effects of extractives on the weight loss by brown rot fungi.**

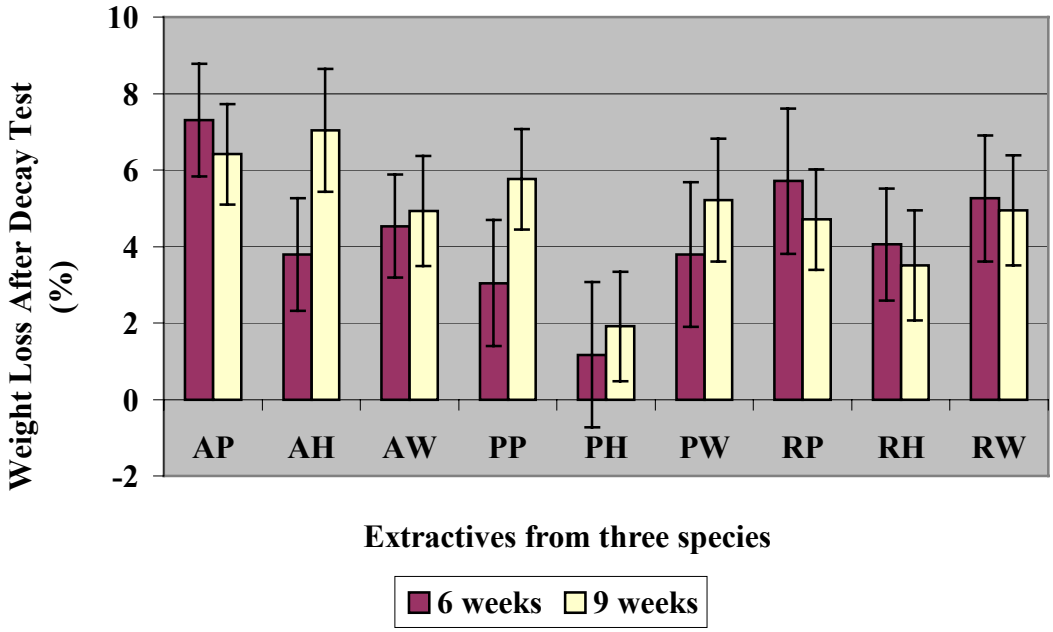



**Figure 6. Effects of extractives on the weight loss by white rot fungi.**

with these two treatments. The weight loss was not significantly different between other paired comparisons. After 9 weeks, the weight loss of the blocks treated with hexane-acetone extractives from POC was significantly lower than those treated with pentane extractives from AYC, hexane-acetone extractives from AYC and pentane extractives from POC. No significant difference was observed among paired comparisons between others.

## 4.2 Comparison of Different Parts of Eastern Red Cedar

### 4.2.1 Segregation of the Different Parts

Branches of an ERC free growing in Baton Rouge, Louisiana, were collected and segregated into three parts: needles, bark, and stem wood. Figure 8 shows the samples before (a) and after (b) the segregation. The needles were dark green, the bark was brown and the stems were yellow and white.


(a) (b)

**Figure 7. Branches of ERC and the different tree parts.**

Table 6 Weights and percentage of the different tree parts.

| Parts | Fresh Weight (g) | Weight Percent (%) | Dried Weight (g) | Water Content (%) | Dry Weight Percent (%) |
|---|---|---|---|---|---|
| Needles | 135.27 | 77.91 | 55.96 | 58.63 | 71.26 |
| Bark | 20.83 | 12.0 | 12.05 | 42.15 | 15.34 |
| Stem | 17.53 | 10.09 | 10.52 | 39.99 | 13.4 |
| Total | 173.63 | | 78.53 | | |

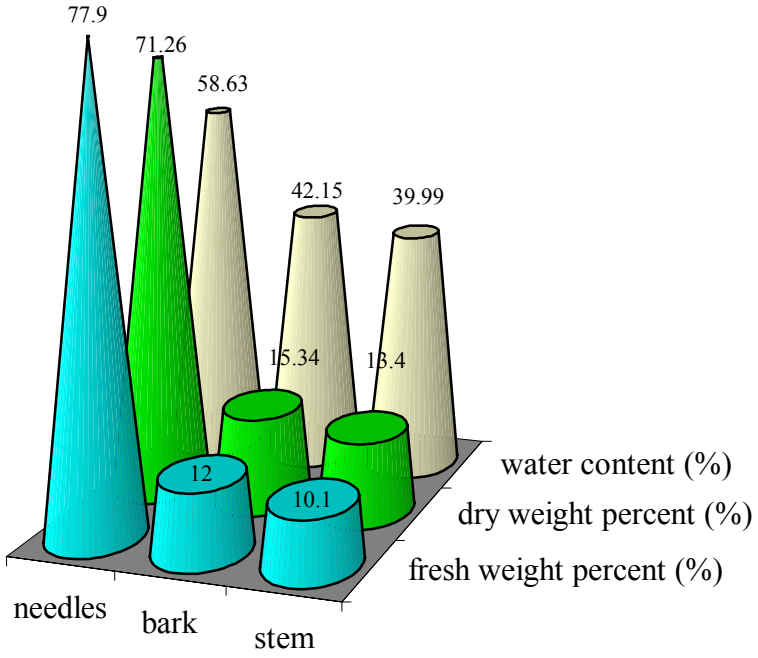



**Figure 8. Weight percent and moisture content of different parts of Eastern red cedar branches (%).**

In these branches, 135.27 grams of needles, 20.83 grams of bark, and 17.53 grams of stems were separated, which were 77.91%, 12.0% and 10.09% of the total fresh weights, respectively. After vacuum drying, the weight percentages of the needles, bark and stem were 71.26%, 15.34% and 13.4%, respectively. Most of the branch samples were needles, and the stem occupied the least proportion. Table 6 and Figure 8 show the sample properties of the different parts.

### 4.2.2 Extractives from Different Tree Parts

The three different tree parts were subjected to consecutive extraction by hexane and acetone. Figure 9 shows the extractives from the three parts. As we can see in the pictures, both of the extractives from the needles by hexane and by acetone (#2, 13) were dark yellow green, in which we believe it contained much chlorophyll, and more chlorophyll was extracted by acetone. The extractives from the bark by hexane (#4) were light yellow green, and the color of those extracted by acetone (#11) was darker, which shows that more chlorophyll was extracted by acetone. They had lighter color than those from the needles, which was in accordance with the color of the bark versus the needles. The extractives from the stem by hexane were yellow oil (#6) and those by acetone (#9) were red oil with green edge around the central oily part.


(a) Hexane Extractives (b) Acetone Extractives

**Figure 9 Extractives from the three parts of Eastern red cedar.**
( #2,13 : from the needles; #4, 11 : from the bark; #6, 9: from the stem)

The extractives were weighed after the solvents were removed. Table 7 and Figure 11 shows the amounts of the extractives.

From Table 7, hexane extractives were 6.49±0.63% from the needles, 4.75±0.21% from the bark and 0.66±0.05% from the stem. Acetone extractives were 6.80±0.61%

from the needles, 5.83±0.30% from the bark and 1.34±0.22% from the stem. Both hexane and acetone removed greater amount of extractives from the needles and the bark than from the stem.

Table 7 Amounts of the extractives from different tree parts.

| | Needles | Bark | Stems |
|---|---|---|---|
| Hexane extractive (%) | 6.49 | 4.75 | 0.66 |
| Standard Deviation (%) | 0.63 | 0.21 | 0.05 |
| Acetone extractive (%) | 6.8 | 5.83 | 1.34 |
| Standard Deviation (%) | 0.61 | 0.30 | 0.22 |

Figure 10 gives a more direct comparison of the extractive amount from these three parts and by the two solvents. These results indicate the difference of organic solvents extractable compounds in the three parts from the branches of Eastern red cedar. More

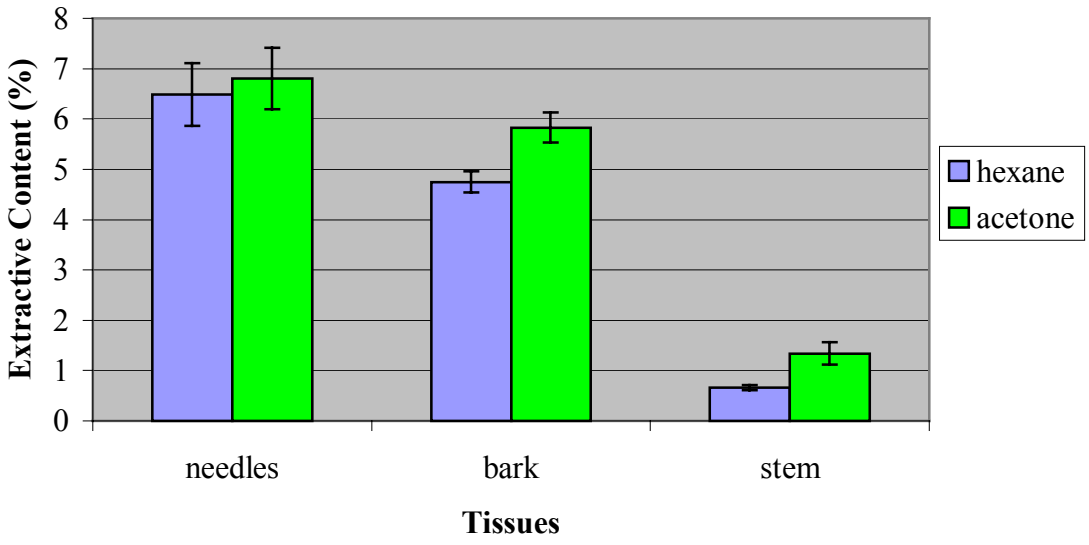



**Fig. 10 Extractive content from different tissues.**

components with low polarity (extracted by hexane and acetone respectively) were extracted from the needles and bark, which suggested the accumulation of such secondary metabolites with small molecular weight during the growth of the tree. A

relatively low percent of extractives were removed from the stem by hexane and acetone, which may be a result of the production and accumulation of high polarity compounds or synthesis of compounds with large molecular weight, or difficult access to the inner side of the cell for the organic solvent.

### 4.2.3. TLC Results of the Extractives from Different Tree Parts

Each extractive was applied to the thin layer chromatography. The developing plate was Silica gel IB2-F and the flowing phase was the mixture of hexane and acetone (v/v= 92.5/7.5). Figure 11 shows the TLC results of the hexane extractives, and the extractives in Figure 12 were extracted by acetone. From Figure 11, we can see that there were at least ten components detected in the needles extractives, twelve components in the bark extractives, and eight components in the stem extractives. However, in Figure 12, only 3 spots were visualized in the extractive from the needles, 2 in the extractives from bark and no components were flowed from the origin point of the extractives from stem. This result indicates that most of the non-polar or low-polar components in the needles and the bark were extracted by hexane and only a small amount of these components were extracted by acetone. It seems most of the low-polar compounds in stems were extracted by hexane, and those extracted by acetone can't be separated further with this flowing phase on these plates.

For easier comparison in further tests and to distinguish the difference between different spots eluted from different parts, we labeled the spots visualized on the plates and calculated their Retention factor value. Table 8 shows the Rf values of these spots. Spots # in Table 8 is same as those labeled in Figure 10 and Figure 11.

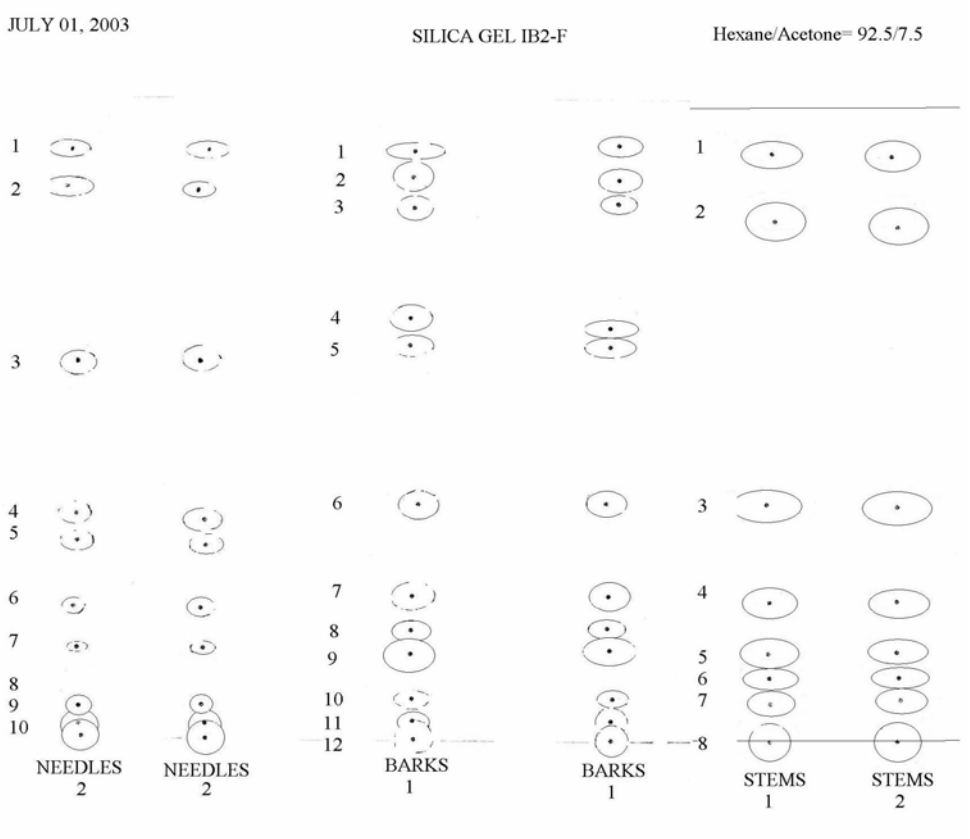
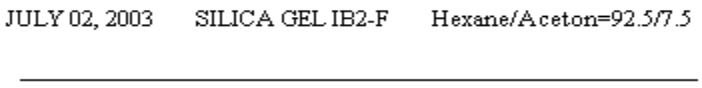



**Figure 11. TLC results of the hexane extractives.**



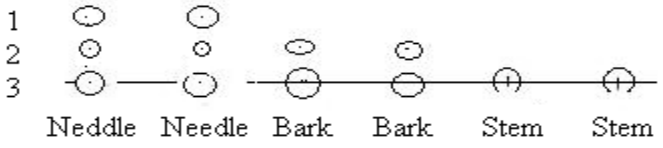




**Figure 12 TLC results of the acetone extractives.**

Table 8 Retention factor (Rf) values of the eluted components.

| Spots # | Hexane Extractives | | | | | |
|---|---|---|---|---|---|---|
| | Needles | | Bark | | Stem | |
| | Rf values | Stdev | Rf values | Stdev | Rf values | Stdev |
| 1 | 0.922 | 0.006 | 0.921 | 0.005 | 0.921 | 0.007 |
| 2 | 0.865 | 0.005 | 0.876 | 0.506 | 0.819 | 0.010 |
| 3 | 0.608 | 0.019 | 0.824 | 0.006 | 0.367 | 0.005 |
| 4 | 0.348 | 0.008 | 0.635 | 0.013 | 0.220 | 0.127 |
| 5 | 0.309 | 0.007 | 0.609 | 0.010 | 0.142 | 0.082 |
| 6 | 0.210 | 0.006 | 0.361 | 0.007 | 0.109 | 0.063 |
| 7 | 0.143 | 0.004 | 0.219 | 0.005 | 0.074 | 0.043 |
| 8 | 0.052 | 0.002 | 0.172 | 0.004 | 0 | |
| 9 | 0.029 | 0.003 | 0.134 | 0.003 | | |
| 10 | 0 | | 0.066 | 0.004 | | |
| 11 | | | 0.030 | 0.004 | | |
| 12 | | | 0 | | | |
| | Acetone Extractives | | | | | |
| 1 | 0.155 | 0.014 | 0.070 | 0.005 | 0 | |
| 2 | 0.055 | | 0 | | | |
| 3 | 0 | | | | | |

Further statistical analysis was applied for the comparison of the Rf values. Table 9 shows the p-values of the paired comparisons of those spots in the hexane extractives having no significant differences with Tukey-Kramer comparison.

Table 9 p-values of paired comparisons that have no significant difference.

| | 1 | C1 | | | 0.286 | C3 | 1 | C4 | 1 | C5 | 1 | C7 | | 1 | C2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B1 | | B2 | B5 | B6 | | B7 | | B9 | | B10 | | B11 | B3 | |
| A1 | 1 | 1 | | | | | | | | | | | | | |
| A2 | | | 0.9887 | | | | | | | | | | | | |
| A3 | | | | 1 | | | | | | | | | | | |
| A4 | | | | | 0.531 | 0.035 | | | | | | | | | |
| A6 | | | | | | | 0.973 | 0.998 | | | | | | | |
| A7 | | | | | | | | | 0.983 | 1 | | | | | |
| A8 | | | | | | | | | | | 0.395 | 0.082 | | | |
| A9 | | | | | | | | | | | | | 1 | | |

Note: The p-value of the comparison between A2 and B2 is 0.9887. The p-value of the comparison between B6 and C3 is 0.286. A, B and C denote the hexane extractive from the needles, bark and stem respectively.

With the comparison of the Rf value of each spot, we found some of the spots may indicate the same component. The p-values of those pairs showing no significant difference at 95% confidence interval in the Tukey-Kramer comparison are listed in Table 9, which indicated there was no significant difference between them and they were labeled with the same color of the circle point in Figure 13.

In total, there may be 12 kinds of compounds detected in the extractives from the branches of ERC. In the three different parts of ERC, the bark yielded the highest mean value by using hexane. It is probable that there were three components in the bark absent in the extractives from the needles (bark #3, 4 and 8) and four absent in the stems (bark #2, 4, 5, 8). And there was one component in the needles and one in the stems, which were not detected in the components in the bark (needle #5 and stem #6).

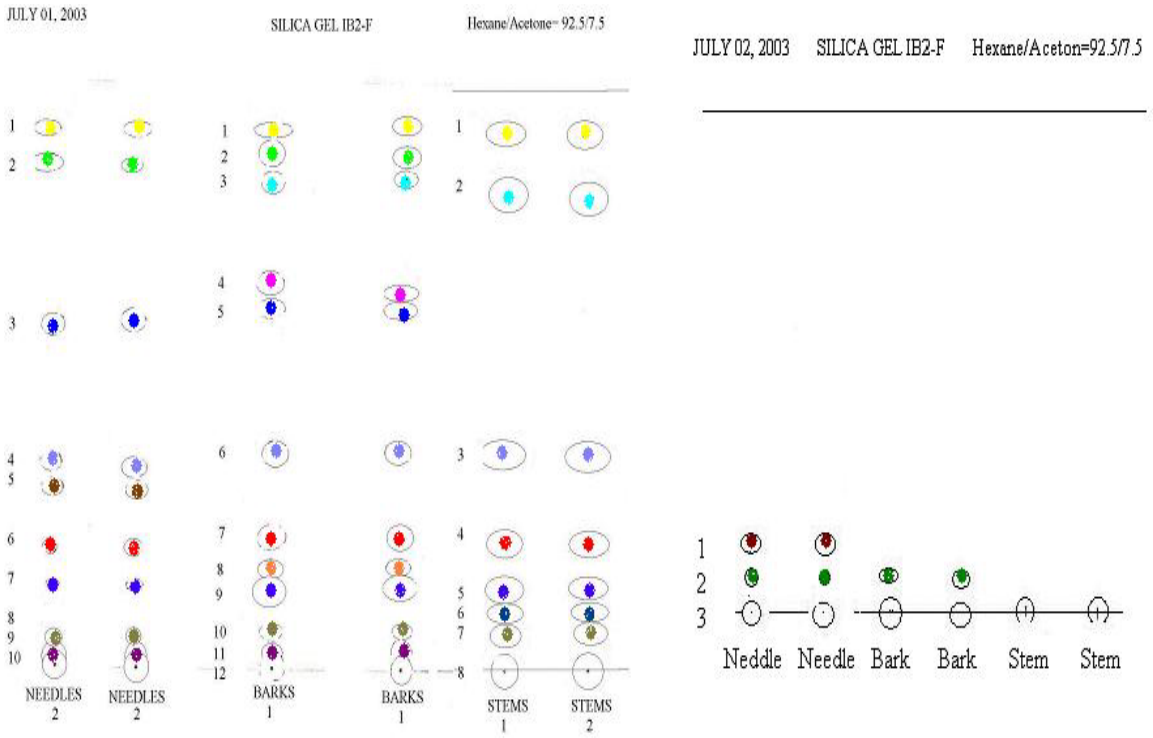



**Figure 13 Result of the comparison of different spots.**
(The same color indicates the Rf values of the spots have no significant difference with a 95% confidence interval.)

### 4.2.4 GC-MS Results of the Hexane Extractives from Needles

For further detection of the known termiticidal compounds in the needles, we applied hexane extractives to a GC-MS system. The Gas Chromatography spectrum is shown in Figure 14. As the spectrum shows, there are more than 15 peaks that were detected in the fraction when the heating speed was set at 5 degrees per minute.

In Figure 14, we didn't find the desired termiticidal compounds, cedrol or widdrol. The detailed spectrum between scan number 3600 to 4200 is shown in Figure 15. We found a peak at around scan number 3900 in other experiments, which may be cedrol, one of the termiticidal compounds in ERC.

However, we detected another compound at scan number 3631, longifolene, which is synthesized from farnesyl diphosphate, the same precursor as usually biosynthesized in plants as cedrol and widdrol (Figure 15). This compound, whose mass spectrum and the fit search result in the library of Terpene was shown in Figure 16, had a score over 900,

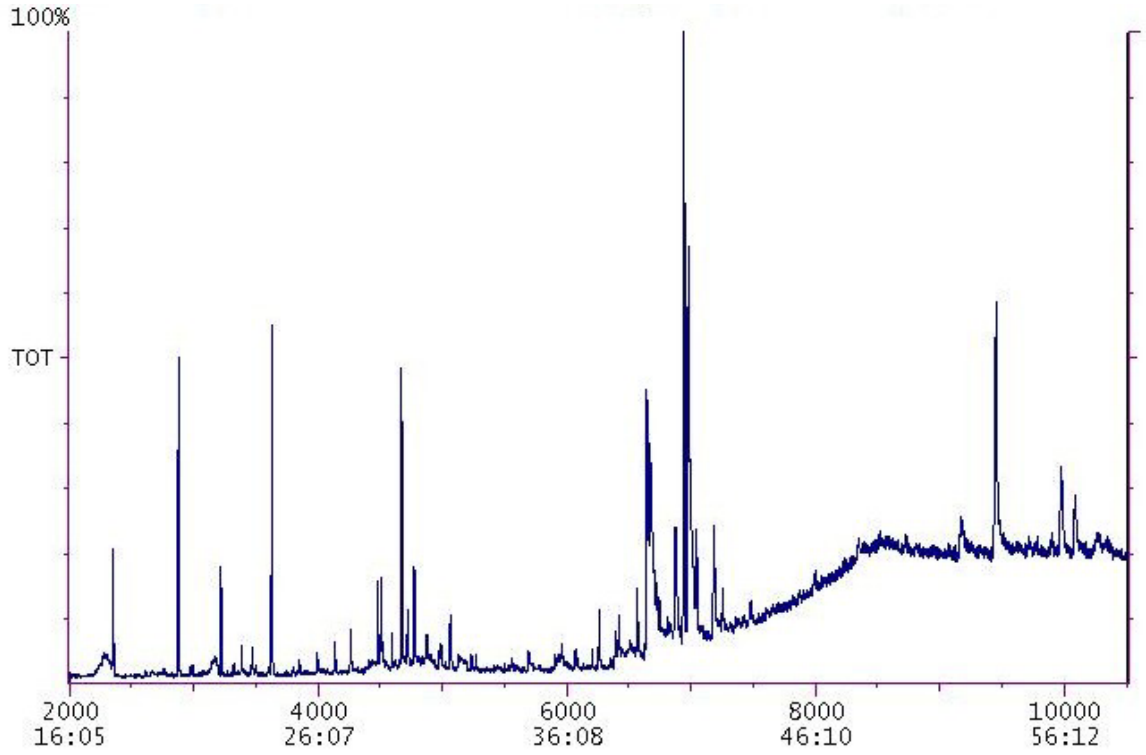



**Fig. 14 GC Spectrum of the hexane extractives from needles of ERC.**

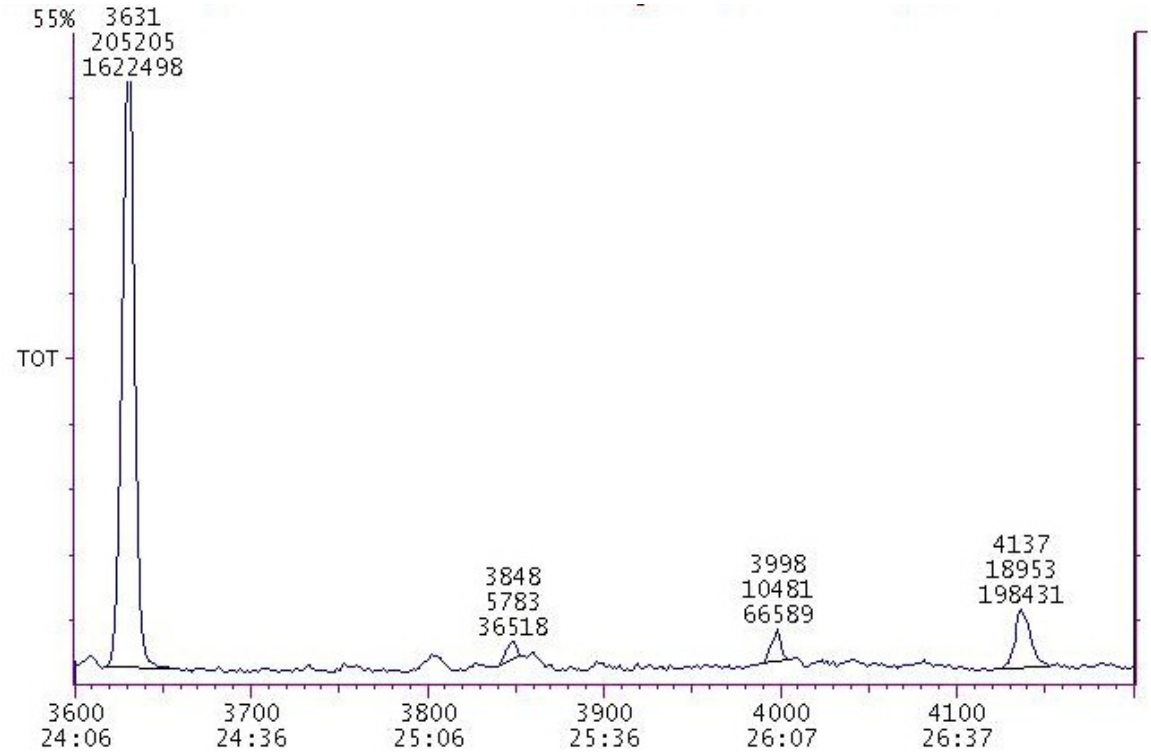



**Figure 15 GC Spectrum of hexane extractives from needles (Scan #: 3600-4200).**

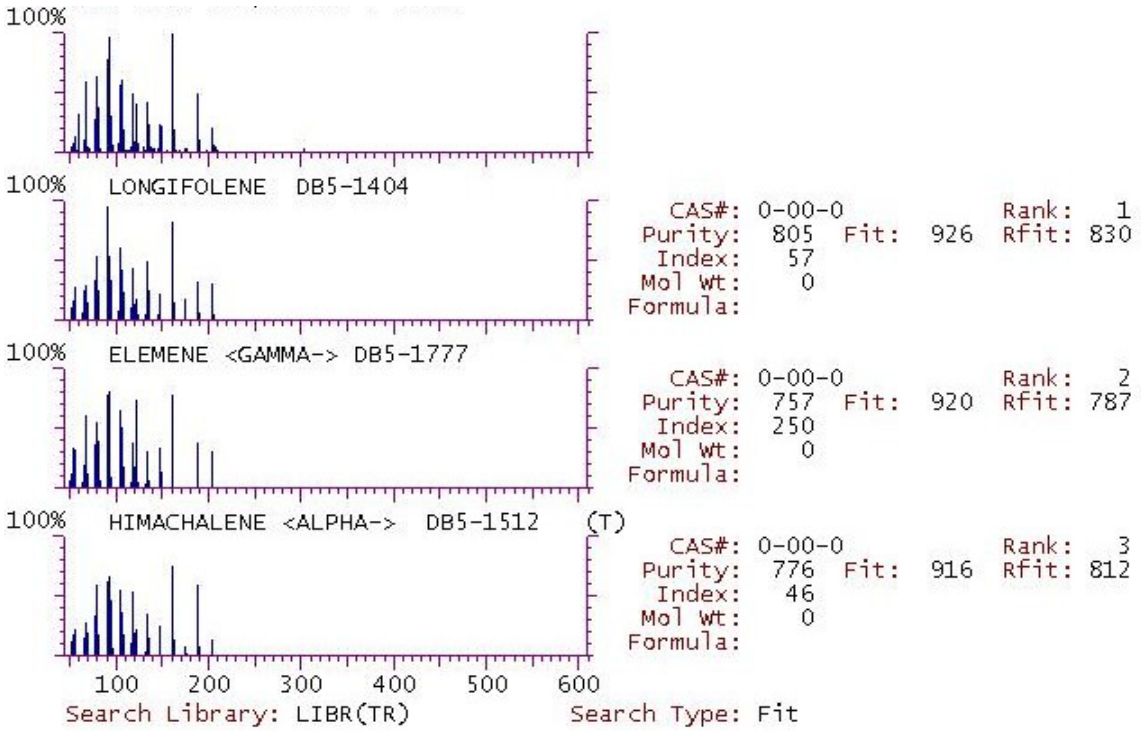



**Figure 16. Search results of mass spectrum of peak at scan #3631.**

indicating that it is very possible to be longifolene. When integrated from scan number 2000 to 10500, the area of this compound is 1,622,498 (totally 52,219,836), which is about 3.11% of total hexane extractives. These results may indicate that the longifolene was synthesized prior to the synthesis of cedrol and widdrol during the growth of Eastern red cedar.

## 4.3. Comparison of the Components in Callus and Seedlings

### 4.3.1 Optimization of the Sterile Time

After cut from the seedlings, the branches have to be sterilized before being cultivated on the medium. Usually they are sterilized in bleach solution at lower concentration. However, too long impregnation or higher concentration will inhibit the growth of the explants. In our test, we sterilized the explants with 70% ethanol, and 1% and 10% bleach; and examined the effects of several combinations of them. The results are shown in Table 10. During the sterilization, two drops of Tween 20 were added to the solution to reduce surface tension and allow better surface contact.

Table10 Effects of sterilization methods on the contamination rate of the explants.

| Methods | 70% ethanol (Seconds) | 1% bleach * | 10% bleach * | Contamination (%) |
|---|---|---|---|---|
| 1 | 5 | 10 Min. | 10 Min. | 82% |
| 2 | 15 | 1 Hour | 15 Min. | 63% |
| 3 | 20 | 3 Hour | 20 Min. | 18% |
| 4 | 30 | 5 Hour | 30 Min. | 10% |

From the results in Table 10, contamination rates dropped as the immersion time increased. The contamination rate was about 10% when treated with Method 4 and 18% with Method 3. Both the results of the contamination rate are satisfactory for our experiment. However, the explants almost stopped growing in Method 4 and they grew normally in Method 3. So, we adopted Method 3 for our tests.

### 4.3.2. Comparison of Two Media on Growth Rate of the Explants

Two kinds of medium (B5 and WPM) were employed in our experiments to cultivate the explants. With 3.1 g/L B5 in the medium, we got a higher average growth rate than the WPM medium at the concentration of 2.3 g/L. The results are shown in Figure 17.

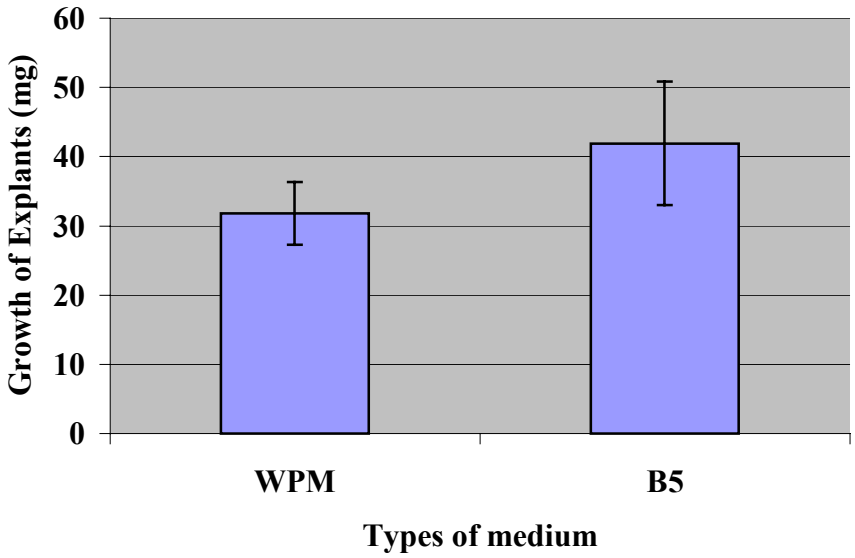



**Figure 17. Effects of medium on the growth of explants after 27 day cultivation.**

From the comparison of the growth of the callus, no significant difference was found for B5 and WPM medium after being cultivated for 27 days. However the mean growth on the B5 medium was higher than that with the WPM medium.

### 4.3.3. Effect of Auxin Concentration on the Growth Rate of the Explants

In order to find out the optimum auxin concentration for the induction of callus, we added 2, 4- D to the medium at three levels (0, 2, 8 mg/L) on both of the media.

The growth rate of the callous increased as the concentration of the auxin increased in both of the medium as shown in Figure 18. However, the growth rate of the explants in B5 medium was much higher than that in WPM medium in every level of the auxin concentration. Compared with the control samples, the explants' growth rate in WPM

medium increased 22.9% and 53.3% with the auxin level at 2 mg/L and 8 mg/L, respectively. And the explants' growth rate increased 32.68% and 35.8%, respectively, when 2 mg/L and 8 mg/L 2, 4-D were added in the B5 medium.

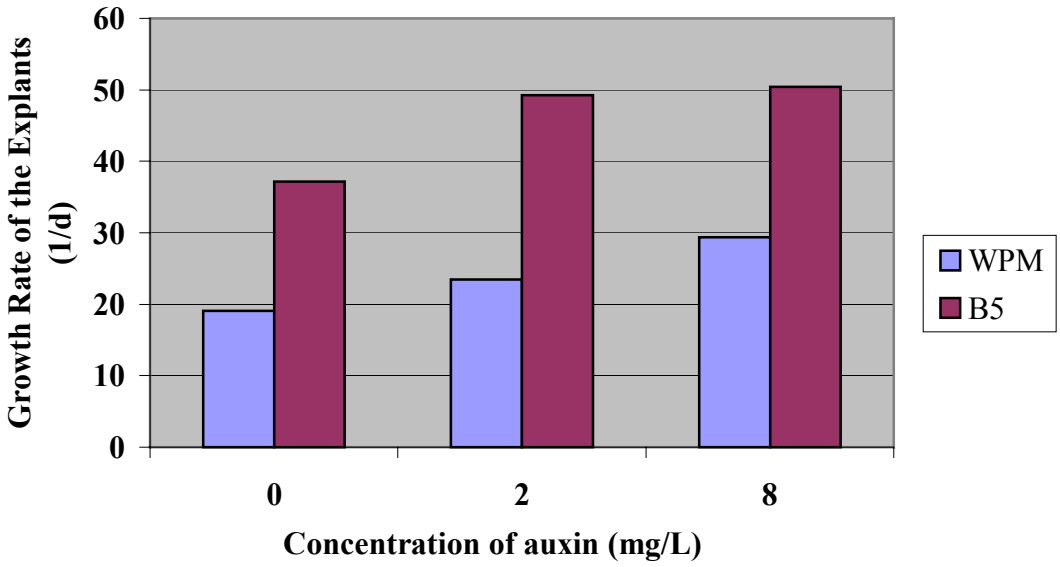



**Figure 18. Effect of auxin on the growth rate of the explants after 2 weeks' cultivation.**

### 4.3.4 Comparison of Extractives from Seedlings and Tissue Callus

Freeze dried callus powder (22.0933 grams) and seedlings powder (22.2146 grams) were collected and soxhlet extracted by hexane at 75 °C. Totally, 0.0666 grams (0.301%) and 0.0534 grams (0.24%) of extractives were removed from the callus and seedlings, respectively. They were dissolved in 4 ml acetone and filtered with a micro syringe and kept in a refrigerator for further analysis. Figure 19 shows the callus after 9 months cultivation and the extractives from the callus and seedlings.

As shown in Figure 19, after cultivated on the WPM and B5 media for 9 months, the explants were dried and became yellow. And callus cells were light green and formed a compact pile on the medium. The original seedling and the callus were collected and

dried in a freeze vacuum drier. Both of them appeared pale white. When they were extracted, the extractives appear to be dark yellow in acetone. However, the extractives from the seedlings were light yellow and were very distinctive from the callus extractives.


**Figure 19. Callus samples and hexane extractives from callus and seedlings.**
(left) Callus in jars, (right) Hexane extractives from callus and seedling

### 4.3.5. TLC Results

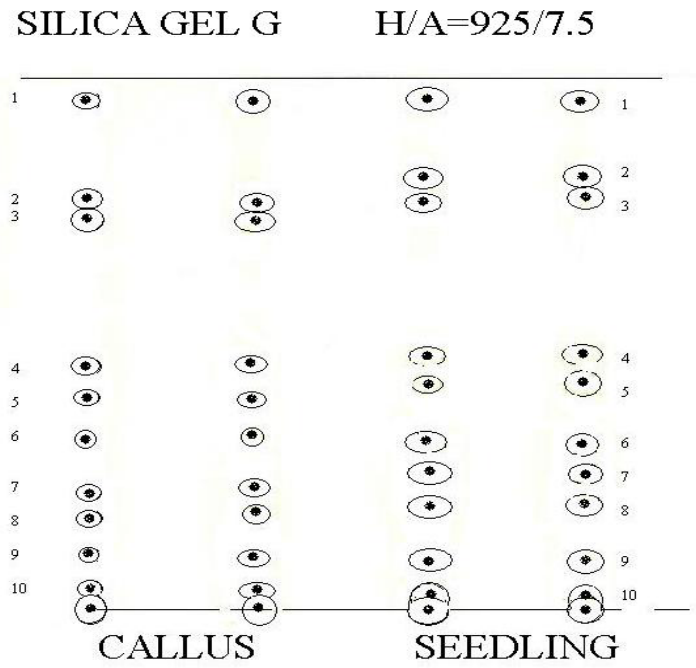



**Figure 20 TLC results of the hexane extractives from callus and seedlings.**

About 30 µl of each extractive solution for each replicate was developed on the Silica G gel plate. 0.5% (w/v) Iodine solution (in chloroform) was sprayed on the plate to detect the compounds flowed from the solution. From Figure 20, we found ten spots were detected in the callus of the tissue culture and ten components in the seedling extractives, besides the original spots, which do not move in this flowing phase. It seems the two resources had little difference in the components they contain. Spot #3 in callus was not detected in the seedlings, and spot #2 in seedlings was absent in the callus. Spot #2 in callus may be the same compound as spot #3 in seedlings and spots #4 to #10 may have little difference from those corresponding spots shown in extractives from the seedlings.

**4.3.6. GC-MS Results**

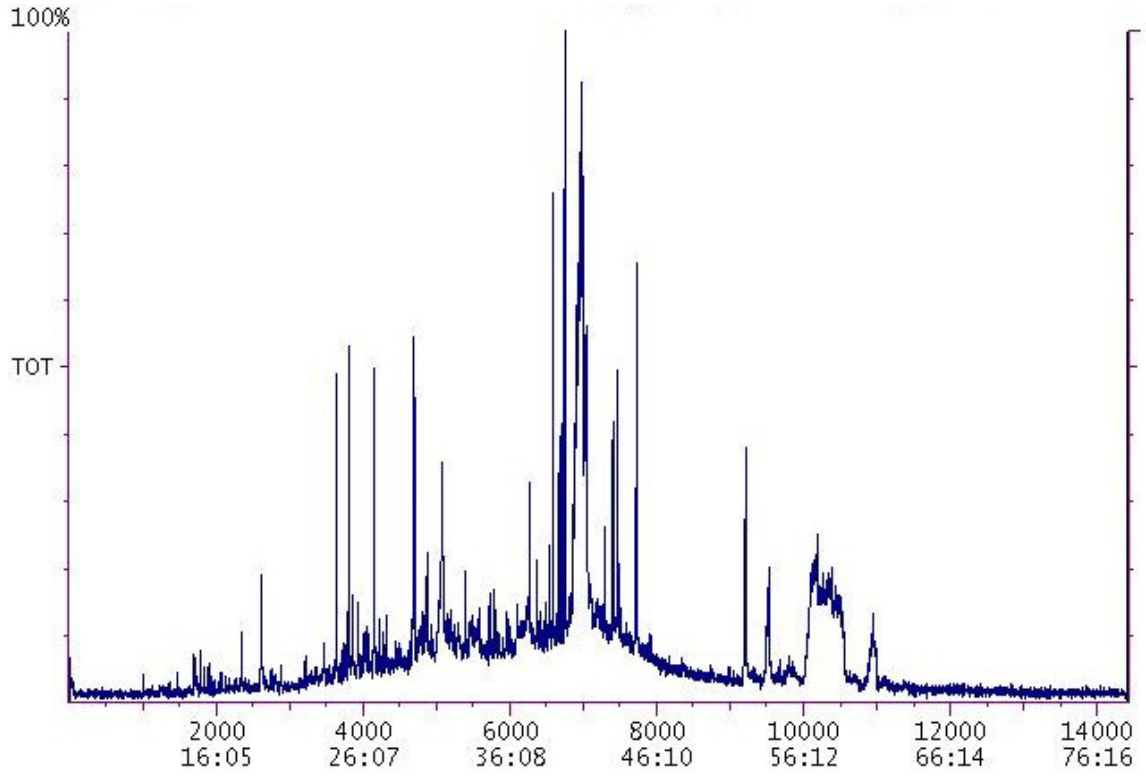



**Figure 21. GC Spectrum of the extractives from seedlings.**

Extractives from the seedlings and callus by hexane were applied to GC-MS for further comparison of the termiticidal compounds. Figure 21 and Figure 22 show the spectrum of the extractives from these two origins.

From the spectrum in Figure 21 and Figure 22, we detected more peaks in the extractives from the seedlings, although the highest peak had lower peak intensity than that in the spectrum of the extractives from the callus. Components in extractives from the seedlings had lower density, and relatively small differences were observed from others. However, the height of the peaks in the spectrum of the extractive from the callus changed much from peak to peak. The peaks at scan # 6727 and # 10210 are much higher than others, which indicate that these two components have higher concentrations than others.

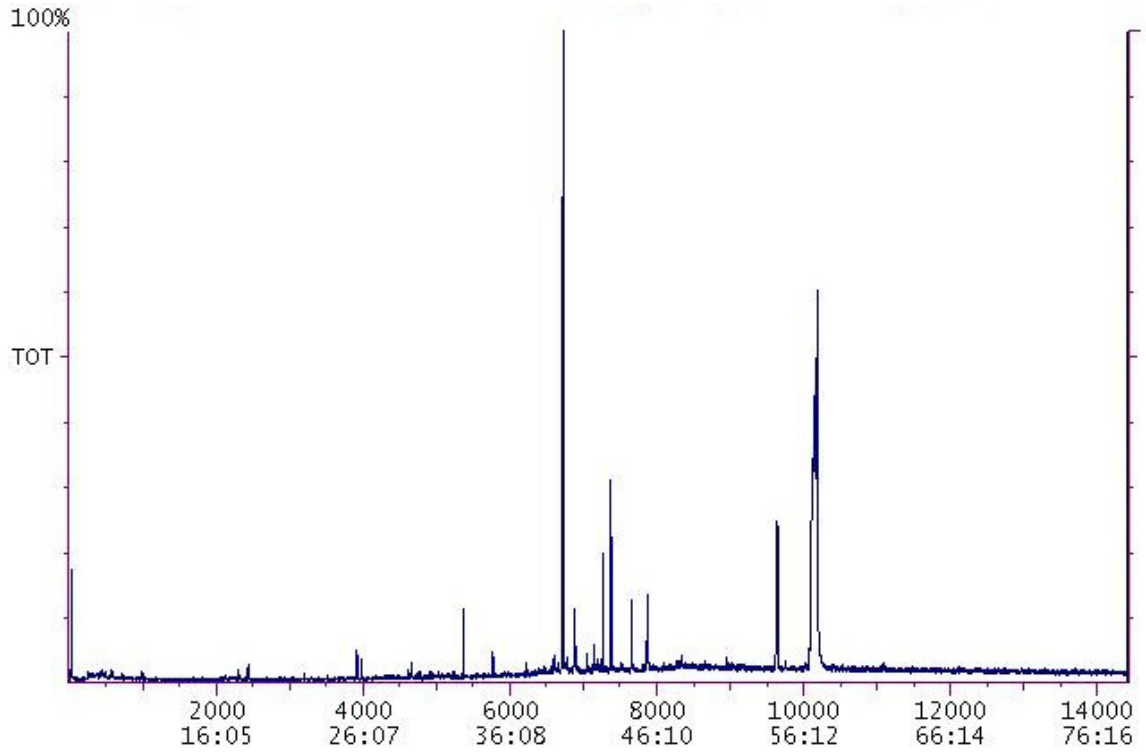



**Figure 22 GC spectrum of the extractives from Callus.**

Figures 23 and 24 show the GC Spectrum from scan number 3600 to 4200 of the extractives from both resources. As shown in the spectrum, we detected cedrol in the seedling extractive at scan number 3941 in Figure 24, whose mass spectrum and search results are shown in Figure 25. We also detected cedrol in the extractives form callus at scan number 3920 in Figure 24, whose mass spectrum and search result in the library of Terpene are shown in Figure 26.

From the calculation of the proportion of the area after integration, the cedrol was about 0.28% in seedlings extractive and 0.4% in the callus extractives. And as we can see in these two pictures, there are only two peaks detected in the callus and over seven peaks were detected in the seedlings.

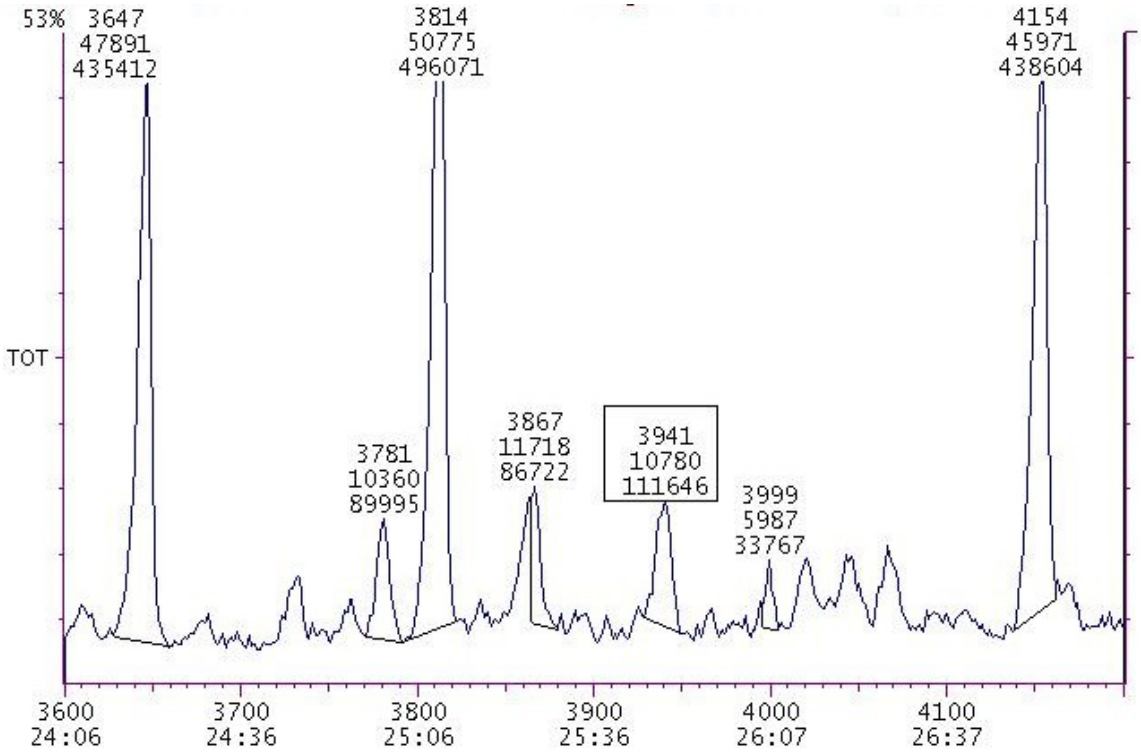



**Figure 23. GC Spectrum of the extractives from seedling (scan #: 3600 to 4200).**

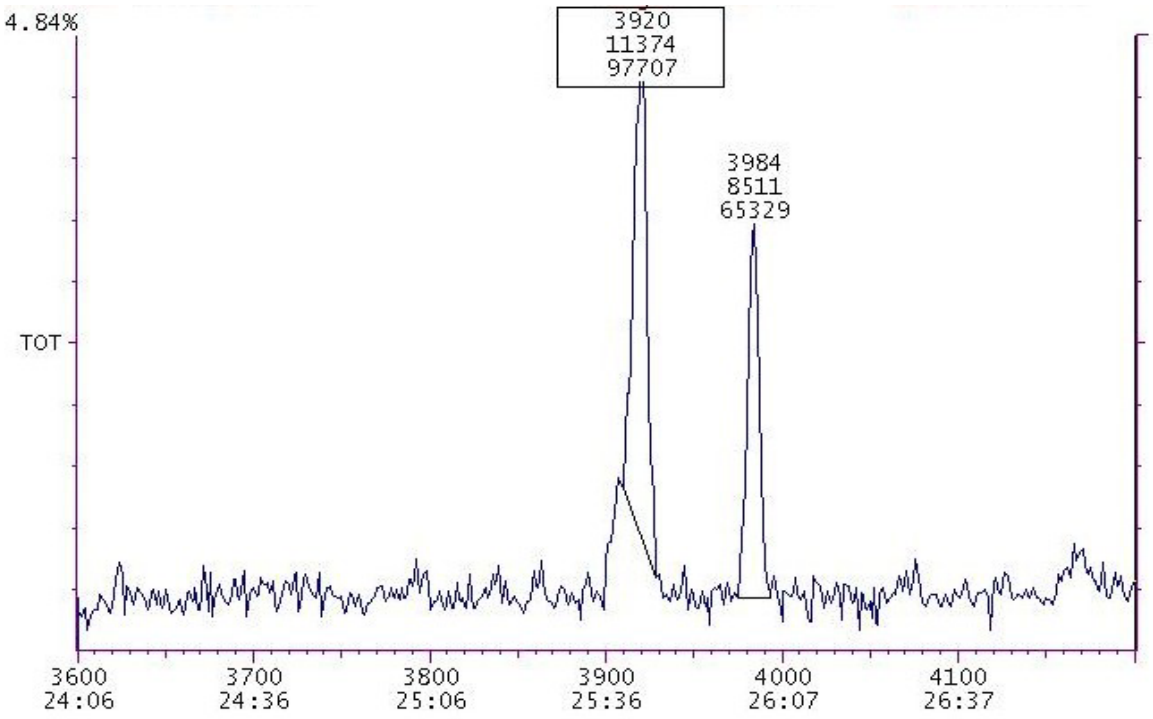



**Figure 24. GC Spectrum of the extractives from the callus (scan #: 3600 to 4200).**

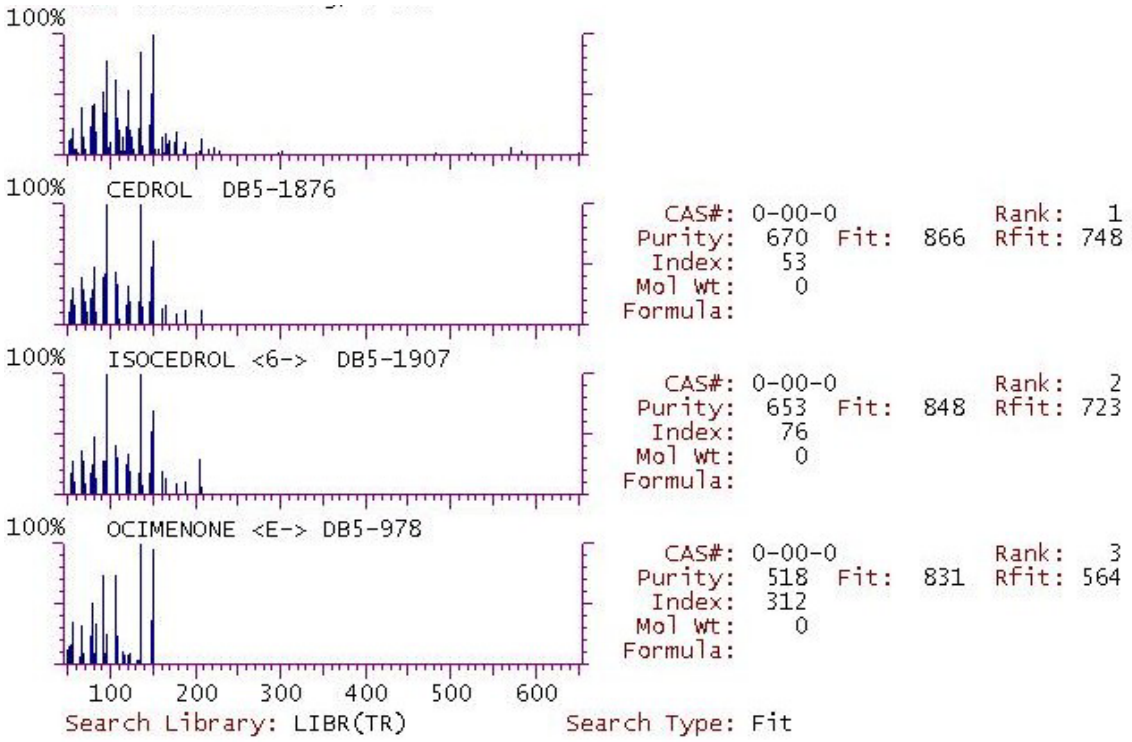



**Figure 25. Mass spectrum of peak 3942 (seedling) and search results.**

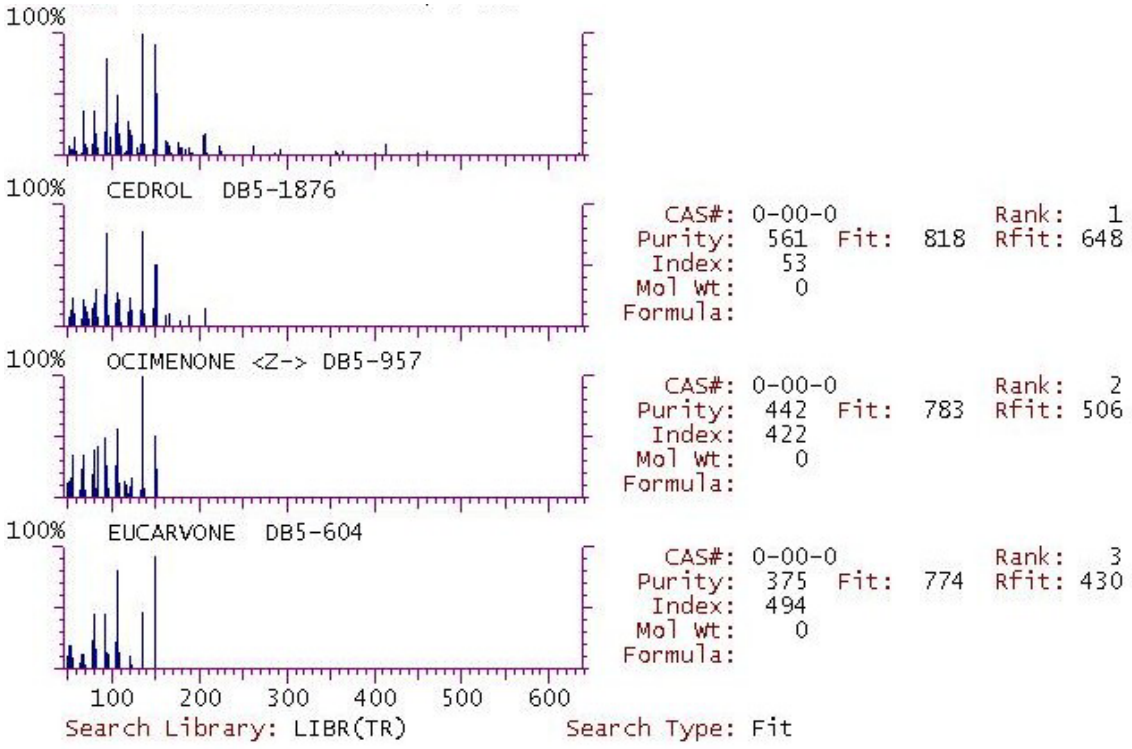



**Figure 26. Mass spectrum of peak 3920 (callus) and search results.**

Another compound detected in the seedling, longifolene, which was detected at scan number 3647 (Figure 23), was about 0.64% of the seedling extractives. This result is lower than that detected in the needles as discussed in Section 2 of Chapter 4.

# CHAPTER 5. SUMMARY AND CONCLUSIONS

Pentane and Hexane-Acetone extractives from Alaska yellow cedar and Port-Orford cedar, and pentane extractives from Eastern red cedar had better inhibition results on the feeding of termites. The strips treated with 5000 ppm pentane extractives from ERC, had significantly lower weight loss than those treated with solutions of other pentane extractives from these three species of wood at this concentration or lower concentrations. Wood blocks treated with pentane extractives from ERC also exhibited significant lower weight loss than other fractions from ERC and from the other two species at 5000 ppm in brown rot decay test. Hexane-acetone soluble extractives exhibited higher antifungal effects than others after 6 or 9 weeks' test with white rot fungi.

From the comparison of the termiticidal components in the different parts in Eastern red cedar, there existed some difference between the needles, bark and the stem of ERC. Cedrol or widdrol were not detected in the extractives from the needles of ERC by hexane.

A small difference was found between the components in ERC callus and the original seedlings from the TLC spectra. The GC-MS spectrum showed fewer types of components and more differences of the peak areas between components in the callus. A higher concentration of cedrol was detected in the callus than that in the original seedlings. Widdrol was not detected in both resources.

# REFERENCES


American Wood-Preservers' Association. 2000. Book of Standards. AWPA. P.O. Box 286, Woodstick, MD. 397-407

Becker, G., Petrovitz, H. J., and Lenz, M. 1971. Über die Ursache der abschreckenden Wirkung von Kiefernholz auf Termiten. Z. angew. Entomol. 68: 180-186

Behr, E.A., Behr, C.T., and Wilson, L.F. 1972. Influence of wood hardness on feeding by the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 65: 457-460

Browning, B.L. 1963. The chemistry of Wood. Interscience Publishers, a division of John Wiley & Sons, New York, London. 607

Bultman, J.D., Raymond H. Beal, Frederick F. K. Ampong. 1979, Natural Resistance of Some Tropical African Woods to Coptotermes formosanus Shiraki, Forest Products Journal, 29(6): 46-51

Canel, C., 1999. From genes to Phytochemicals: the Genomics Approach to The Characterization and Utilization of Plant Secondary Metabolites. Proc. WOCMAP-2, Biological Resources, Sustainable Use, Conservation, Ethnobotany, Eds. N. Caffini et al. Acta Hort. 500. ISHS. 51-56

Carter, F. L., and Smythe, R.V. 1974. Feeding and Survival Responses of *Reticulitermes falvipes* (Kollar) to Extractives of Wood from 11 Coniferous Genera. Holzforschung, 28(2): 41-45.

Carter, F.L., Allison M. Garlo, and James B. Stanley. 1978. Termiticidal Components of Wood Extracts: 7-Methyljuglone from *Diospyros virginiana*, Journal of Agric. Food Chem., 26(4): 869-873

Carter, F. L.; Huffman, J. B. 1982. Termite responses to wood and extracts of melaleuca, Wood Science, 14(3): 127-133

Chang, S.H., Ho, C.K., Tsay J.Y. 1996. Callus Initiation, Growth, and Taxol Production of *Taxus mairei*. Taiwan Forest Science. 11(4): 445-453

Chang, S.T., Cheng SenSung, Wang ShengYang. 2001. Antitermitic activity of essential oils and components from Taiwania (Taiwania cryptomerioides), Journal of Chemical Ecology, 27(4): 717-724

Chang, S. T., Wang, S. Y., Kuo,Y. H. 2003. Resources and Bioactive Substances from Taiwania (*Taiwania cryptomerioides*). Journal of Wood Science. 49: 1-4.

Chen, J.; Henderson, G.; Laine, R.A. 1988. Isolation and Identification of 2-Phenoxyethanol from a Ballpoint Pen Ink as a Trail-Following Substance of Coptotermes formosanus Shiraki and Reticulitermes sp.. Journal of Entomol. Sci., 33 (1): 97-105.

Chen, J., Gregg Henderson. 1996. Determination of Feeding Preference of Formosan Subterranean Termite (Coptotermes formosanus Shiraki) for some Amino Acid Additives, Journal of Chemical Ecoolgy, 22(12): 2359-2369

Chen, J., Gregg Henderson, et al. 1998. Naphthalene in Formosan Subterranean Termite Carton Nest, Journal of Agric. Food Chem., 46(6): 2337-2339

Cornelius, M.L., J.Kenneth Grace, Julian R. Yates III. 1997. Toxicity of Monoterpenoids and Other Natural Products to the Formosan subterranean Termite (isoptera: Rhinotermitidae), Journal of Economic Entomology, 90(2): 320-325

Erdtman, H. and Hirose, Y. 1962. The Chemistry of the Natural Order Cupressales. ACTA Chemica Scandinavica, 16: 1311-1314

Ferreira, J. F. S., and Duke, S. O. 1997. Appraches for Maximising Biosynthesis of Medicinal Plant Secondary Metabolites. AgBiotech Newsand Information, 9(12): 309-316

Franzios, G., Maria Mirotsou, Emmanouel Hatziapostolou, Jiri Kral, et al. 1997. Insecticidal and Genotoxic Activities of Mint Essential Oils, Journal of Agric. Food Chem., 45(7): 2690-2694

Fukui, H., Hasan A.F.M. F., Ueoka T., Kyo M. 1998. Formation and Secretion of A New Brown Benzoquinone by Hairy Root Cultures of *Lithospermum erythrorhizon*. Phytochemistry, 47(6): 1037-1039

Grace, J.K., Robin T. Yamamoto. 1994. Natural Resistance of Alaska-cedar, Redwood, and Teak to Formosan subterranean Termites, Forest Products Journal, 44(3): 41-45

Green, F. III, Stan Lebow and Tsuyoshi Yoshimura. 2000. Inhibition of Termite Damage by N/N-napthaloylhydroxyamine (NHA): Reticulotermes flavipes (Kollar) vs. Coptotermes formosanus Shirdaki, IRG/WP 00-10354

Gupta, B.K.; Sen-Sarma, P. K. 1978. Antitermite properties of some anthraquinone derivatives, Holzforschung und Holzverwertung, 30(3): 57-58

Henderson, G., Jian chen. 1998. Termites Fumigate Their Nests with aphthalene, Nature, 392(4): 558

Hse, C.Y., Tang, R.C. et al. 2002. Bending Properties and Termite-resistance of Flakeboard Made from Wood with High Natural Durability. Proceedings of the

6th Pacific rim Bio-Based Composites Symposium & Workshop on The Chemical Modification of Cellulosics. 1: 308-314

Ho, C.K., Chen, Z.Z., Tsai, J.Y., Yang, J.C. 1997. Nodule Culture of *Paulownia* x *taiwaniana*. Taiwan Forest Science, 12(1): 39-45

Hu Z. B. and Alfermann, A.W. 1993. Diterpenoid Production in Hairy Root Cultures of *Salvia miltiorrhiza*. Phytochemistry, 32(3): 669-703

Ingram L.L. and Templeton M. C. 2000. Knot, heartwood, and extractives related to VOCs From Drying Southern Pine Lumber. Journal of Wood Chemistry and Technology, 20(4): 415-439.

Jain, J. K.; Dobriyal, P. B.; Thapam R. S; Satish Kumar. 1990. Antitermite efficacy tests on chlorinated phenolic compounds, Journal of the Timber Development Association of India, 36(1): 5-12

Kinyanjui, T.; Gitu, P. M.; Kamau, G. N. 2000. Potential antitermite compounds from Juniperus procera extracts, Chemosphere, 41(7): 1071-1074

Laine, R.A., Jian chen and Gregg Henderson. 1998. Isolation and Identification of 2-Phenoxyethanol from a Ballpoint Pen Ink as a Trail-Following Substace of Coptotermes formosanus Shiraki and Reticulitermes sp., J. Entomol. Sci., 33(1): 97-105

Lin, T. 1998. Effects of essential oil from the leaves of seven Eucalyptus on the control of termite, Forest Products Industries, 17(4): 751-760

Lin, T.S. and Chang, T.T. 1998. Anti-termite properties of wood. Bull. Taiwan For.Res. Inst. Ext. Series 22 (in Chinese).

Maistrello, L.; Henderson, G.; Laine, R. A. 2001. Effects of nootkatone and a borate compound on Formosan subterranean termite (Isoptera: Rhinotermitidae) and its symbiont protozoa, Journal of Entomological Science, 36(3): 229- 236

McDaniel, C.A. 1989. Major Termiticidal Components of Heartwood of Port-Orford-cedar, Chamaecyparis lawsoniana (A.Murr.) Parl., Material und Organismen, 24: 1-15

McDaniel, C.A., Klocke, J.A., and Balandrin, M.F. 1989. Major antitermitic wood extractive components of Eastern redcedar, Juniperus virginiana, Material und Organismen, 24: 301-313

McDaniel, C.A. 1992. Major antitermitic components of the heartwood of southern catalpa, Journal of Chemical Ecology, 18(3): 359-369

Obst, J.R. 1998. http://www.fpl.fs.fed.us/documnts/pdf1998/obst98a.pdf. Special (secondary) Metabolites from Wood.

Paes, J. B.; Morais, V. de M.; Lima, C. R. de. 2001. Natural resistance of nine wood species of the Brazilian semi- arid region to subterranean termites, under feeding preference test, Brasil Florestal, 20(72): 59-69

Panshin, A. J., Carl De Zeeuw, Textbook of Wood Technology, third edition. 1971. 496-498

Potter, M. F. 1997. Termites. In: Mallis, A. Handbook of Pest Control. Eighth edition. Maillis Handbook & Technical Training Company. 232-332

Saeki I., Sumimot M., and Kondo T. 1971. The Role of Essential Oil in Resistance of Coniferous Woods to Termite Attack. Holzforschung, 25(27): 57-60

Saeki I., Masashi Suminmoto, et al. 1973. The Termiticidal Substances from the Wood of *Chamaecyparis pisifera* D. Don, Holzforschung, 27(3): 93-96

Sajap, A. S.; Aloysius, F. 2000. Effects of leaf extracts of Azadirachta excelsa on Coptotermes curvignathus (Isoptera: Rhinotermidae), Sociobiology, 36(3): 497-503

Scheffrahn, R.H., et al. 1988. Allelochemical Resistance of Bald Cypress, *axodium distichum*, Heartwood to the Subterranean Termite, *Coptotermes formosanus*, Journal of Chemical Ecology, 14(3): 765-776

Sen-Sarma, P. K.; Gupta, B. K. 1979. Antitermite characteristics of wood of ,Lophopetalum wightianum, Holzforschung und Holzverwertung, 31(5): 100- 101

Sharma, R. N., V. B. Tungikar, P. V. Pawar and P. H. Vartak. 1994. Vapour Toxicity and Repellency of some Oils and Terpenoids to the Termite, Odontotermes brunneus, Insect Sci. Applic., 15(4/5): 495-498

Singh, G.; Singh, O. P.; Rao, P.; Singh, P. K.; Pandey, K. P. 2001. Studies on essential oils, Part 29: Insecticidal activity of some volatile oils and monoterpenoids against white termite (Odontotermes obesus Rhamb.), Sugar Cane International, November, 18-20

Smith, B. C., Starrattt, A. N. and Bodnaryk, R.P. 1973. Oviposition responses of *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae) to the wood and extracts of *Juniperus virginiana* and to various chemicals. Ann. Entomol. Soc. Amer. 66(2): 452-456.

Tellez, M.; Estell, R.; Fredrickson, E.; Powell, J.; Wedge, D.; Schrader, K.; Kobaisy, M. 2001. Extracts of Flourensia cernua (L): volatile constituents and antifungal,

antialgal, and antitermite bioactivities, Journal of Chemical Ecology, 27(11): 2263-2273

Tellez, M., Rick Estell, Ed Fredrickson, et al. 2001. Extracts of *Flourensia cernua* (L): Volatile Constituents and Antifungal, Antialgal, and Antitermite Bioactivities, Journal of Chemical Ecoolgy, 27(11): 2263-2273

Valles, S. M.; Woodson, W. D. 2002. Group effects on insecticide toxicity in workers of the Formosan subterranean termite, Coptotermes formosanus Shiraki, Pest Management Science, 58(8): 769-774

Vanucci, C., C. Lange, G. Lhommet, B. Dupont, et al. 1992. An Insect Antifeedant Limonoid From Seed of Khaya Ivorensis, Phytochemistry, 31(9): 3003-3004

Vidensek, N.; Lim, P.; Campbell, A. and Carlson C. 1990. Taxol Content in Bark, Wood, Root, leaf, Twig, and Seedling from Several *Taxus* species, Journal of natural Products, 53(6): 1609-1610.

Wang, S. F.; Wang, C. L.; Hsieh, T. C.; Chu, C. C. 1987. The antitermite characters of major native woods in Taiwan and the termite-controlling properties of chemicals, Bulletin of the Taiwan Forestry Research Institute, 2(2): 117-127

Wang, S. Y.; Chung, T. Y. 1989. The accelerated laboratory test of the natural antitermite characteristics of eight wood species and five wood preservations, Forest Products Industries, 8(3): 13-31

Wang, Y.N. 1990. Callus Formation and Cell Suspension Culture of *Cunninghamia lanceolata* var. *lanceolata* and *Taiwania cryptomerioides*. Quart. Journ. Expt. Forest, NTU, 4(4): 1-17

Wang, Y.N., Chiang, C.H., Wu, S. H. 1991. The Propagation by Tissue Culture of *Pseudotsuga wilsoniana* Hay. Quart. J. Expt. Forest, NTU, 5(2): 1-15.

Yaga, S. and Kinjo, K. 1985. On the resistance of Okinawan timbers 9. Termiticidal substance from the wood of Tuettarda speciosa L. Mokuzai Gakkaishi, 31: 684-687

Yatagai, M.; Miyazaki, Y.; Morita, S. 1991. Extractives from yakusugi bogwood and their termicidal activity and growth regulation effects on plant seeds, Mokuzai Gakkaishi = Journal of the Japan Wood Research Society, 37(4): 345-351

Zhang, C.P., Li, C., et al. 2001. Effects of Fungal Elicitor on Cell Status and Taxol Production in Cells suspension Cultures of *Taxus chinesis* var *mariei*. Chinese Journal of Biotechnology. 17(4): 436-440

Zhu, B.C. R., Gregg Henderson, Feng Chen, et al. 2001. Nootkatone is a Repellent for Formosan subterranean Termite (coptotermes formosanus), Journal of Chemical Ecoolgy, 27(3): 2001

Zhu, B. C. R.; Henderson, G.; Chen, F.; Fei, H. X.; Laine, R. A. 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite, Journal of Chemical Ecology, 27(8): 1617-1625

# VITA

Yaojian Liu was born on October 1971, Yantai, People's Republic of China. He graduated from high school and enrolled in the Department of Food Engineering in Shandong Institute of Light Industry in 1990. He graduated with a bachelor's degree in bioengineering in 1994. He was employed in Yantai Winery of Ji'nan Railway Bureau from 1994 to 1998. He enrolled in South China University of Technology in September 1998 and graduated with a master's degree in biochemical engineering in April 2001. He began his study at Louisiana State University in January 2002 to pursue his master's degree in forestry. He has done some studies on the termiticidal compounds in some wood species.