Proceedings IRG Annual Meeting (ISSN 2000-8953)

IRG/WP 16-10857

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

GC-MS Characterizations of Termiticidal Heartwood Extractives from Wood Species Utilized in Pakistan

Mark E. Mankowski[1], Blossie Boyd[1], Babar Hassan[2] and Grant T. Kirker[3]

[1]USDA-FS Forest Products Laboratory
Durability and Wood Protection
201 Lincoln Green
Starkville, MS USA 37959

[2]University of Agriculture Faisalabad
Department of Entomology
Jail Road
38000, Punjab, Pakistan

[3]USDA-FS Forest Product Laboratory
Durability and Wood Protection
One Gifford Pinchot Drive
Madison, WI, USA, 53726-2398

Paper prepared for the 47th IRG Annual Meeting
Lisbon, Portugal
15-19 May 2016

IRG SECRETARIAT
Box 5609
SE-114 86 Stockholm
Sweden
www.irg-wp.com

GC-MS Characterizations of Termiticidal Heartwood Extractives from Wood Species Utilized in Pakistan

Mark Mankowski[1], Blossie Boyd[1], Babar Hassan[2], and Grant Kirker[3]

[1]USDA-FS Termite Research Laboratory, Starkville, MS, USA memankowski@fs.fed.us

[1]USDA-FS Termite Research Laboratory, Starkville, MS, USA bboyd@fs.fed.us

[2] University of Agriculture Faisalabad, Pakistan sialuaf@gmail.com

[3]USDA-FS Forest Products Laboratory, Madison, WI, USA gkirker@fs.fed.us

ABSTRACT

Wood species that exhibit innate tolerance to wood destroying organisms such as termites are considered to be naturally durable. This durability can, in part, be due to the complex chemical compounds in the heartwood of naturally durable wood species. We examined the effects of varying concentrations of heartwood extractives on the subterranean termite, *Reticulitermes flavipes* from four wood species from Pakistan (*Dalbergia sissoo, Cedrus deodara, Morus alba* and *Pinus roxburghii*) as well as Teak (*Tectona grandis*). Termites showed increasing levels of mortality with increasing concentration of heartwood extractive when exposed to extractive treated non-durable southern yellow pine (SYP) blocks in a force feeding test compared to SYP blocks treated with water or solvent (ethanol: toluene) only. Characterizations of heartwood extractives were performed using Gas Chromatography-Mass spectrometry (GC-MS). Chemical profiles were prepared for each wood species' extractives and are discussed relevant to their termiticidal properties. Future work will focus on further isolation of bioactive compounds or synergistic groupages of bioactive compounds from these and other wood species for use as environmentally friendly insecticides/termiticides for wood and wood based materials.

Keywords: naturally durable wood, heartwood extractives, *Tectona grandis, Dalbergia sissoo, Cedrus deodara, Morus alba, Pinus roxburghii, Reticulitermes flavipes*, GC-MS

1. INTRODUCTION

Wood species that possess the ability to impede biodeterioration are considered to be naturally durable. Past work identified several factors that impart natural durability including, hardness, density and extractive content (Arango *et al.* 2006, Scheffer 1966). This work noted that extractive content appeared to be the most important factor affecting a species natural durability. Heartwood extractives are non-structural components produced by a living tree in response to wounds, physiological stress, or as waste products. Extractives have been found to be highly variable and consist of a wide range of chemical compounds such as terpenoids, alkaloids, condensed tannins, phenols and a plethora of others (Taylor *et al.* 2002).

Little is known about the biological function and action of many of these compounds. This lack of understanding has impeded acceptance of naturally durable woods in building codes for certain applications such as ground contact (Morris *et al.* 2011). Durability has been found to vary widely both within and between trees of the same species (Imamura 1989). Areas of south Asia, including Pakistan, utilize several naturally durable species in areas of high termite pressure.

The wood species used in this study are commonly available woods in Pakistan that are being evaluated for potential resistance against termites in that region including *Heterotermes indicola*

(Wassman) and *Odontotermes obesus* (Rambur), which are major pests of wood in service in this region (Manzoor and Mir 2010). They are as follows:

- *Tectona grandis* (L) (Teak). Teak wood was used as a durable reference in this study as it has a proven track record of service (DaCosta *et al.* 1958, Scheffer and Morrell 1998) and is also available in Pakistan. Previous studies indicate teak durability is largely due to the presence of quinones in the heartwood (Lukmandaru and Takahashi 2009). Recent reports on environmental impacts of plantation teak production indicate that availability is not an absolute (Pandey and Brown 2000) and that durability varies due to location of origin (Haupt *et al.* 2003) and stand management practices (Bhat and Florence 2003).
- *Dalbergia sissoo (*Roxb) (Shisham). *D. sissoo* grows wild in ditch banks and roadsides and is an important commercial species for production of furniture and other goods in Pakistan (Sheikh 1989, Tewari 1994a). *D. sissoo* is classified as a class 1 (very resistant) wood species based on previous studies (Scheffer and Morrell 1998). Since 2005, *D. sissoo* stands have been experiencing high mortality due to dieback from several suspected pathogens (Khan *et al.* 2000). Past studies have indicated that heartwood extractives of *D. sissoo* contain high levels of stilbenes, polyphenols and quinones (Seshadri 1972).
- *Cedrus deodara* (Roxb G. Don.) (Cedar). *C. deodara* is also an important tree species in Pakistan. *C. deodara* is extremely common in the higher elevations of Pakistan and has been a valued wood for centuries. A wide range of chemical compounds from *C. deodara* have been previously studied and determined to have numerous medicinal properties (Chaudhary *et al*. 2011a). *C. deodara* is listed as moderately resistant (class III) to decay (Scheffer and Morrell 1998).
- *Morus alba* (L) (White Mulberry). *M. alba* is native to China, but is widely distributed in Pakistan and the Himalayan region due to its connection with silk production. *M. alba* is listed as an invasive plant in Pakistan (Qureshi *et al.* 2014), as well as North America as it hybridizes with the native Red Mulberry (*Morus rubra*) and decreases its vigour (Burgess and Husband 2006). Heartwood of *M. alba* is listed as non-resistant (class IV) (Scheffer and Morrell 1998) but has been shown to contain simple phenolics, benzophenones, flavonoids, and other chemical compounds (Rowe and Conner 1979) and has been studied extensively for its nutritional and medicinal properties (Omidiran *et al.* 2012).
- *Pinus roxburghii* (Sarg) (Chir Pine). Chir Pine is a staple wood species in Pakistan. It has a wide range of uses including building material, heating and has published medicinal value (Tewari 1994b). Although *P. roxburghii* is listed as non-resistant (class IV) to decay (Scheffer and Morrell 1998) extracts of this species have been found to have medicinal and antibacterial properties and heartwood extractives of *P. roxburghii* have been reported to contain abietic acid, longifolene and low levels of several stilbenes (Shuaib *et al.* 2013).

More efficient utilization of local trees of Pakistan would provide for potential value added products that could be obtained from mill cull and less desirable parts of the tree that are normally left behind, landfilled or burned. Effective wood utilization is also a prime directive of the US Forest Service in order to remove dead and diseased inventory from otherwise productive managed forest stands. In this study we tested the solvent extracts of the aforementioned wood species against the termite *Reticulitermes flavipes* (Kollar) and performed GC-MS analysis of the extractives in hopes to elucidate on the toxic components of these durable species.

2. EXPERIMENTAL METHODS

2.1 Preparation Wood and Extractives.

Raw lumber of *Dalbergia sissoo, Cedrus deodara, Morus alba* and *Pinus roxburghii* were purchased from a timber market located at Jhang road Faisalabad (Pakistan), and shipped to FPL Starkville, Mississippi (USA). Marine grade *Tectona grandis* (Teak) was acquired from a supplier in the United States (McIlvain, Pittsburg, PA) and used as a durable reference material in this study. Large boards from each species were evened with a planer and cut into 19×19×19 mm blocks. Shavings from this process were used for the preparation of extractives. Air dried wood shavings (12 g) were soxhlet extracted using 300 ml of ethanol: toluene (2:1) as solvent according to ASTM D1105-96 (ASTM 2014). Shavings were added to soxhlets with a small amount of cotton placed below and above to contain the shavings and extracted for a total of six hours. The resulting aliquots were evaporated to dryness at reduced pressure by using a rotary evaporator (BUCHI, Rotavapor R-114) and extraction yield was calculated per gram of wood shavings (Ordonez *et al*. 2006). Stock solutions of 100mg/ml of each extract were prepared by re-imbibing the dry extract with solvent (ethanol: toluene). The extractives were stored in litre jars in darkness at 4°C. Although origin and growth characteristics were not obtained for these woods, mean extractive content was calculated for each wood species and is presented in Table 1 along with other relevant wood characteristics.

Table 1: General characteristics of the wood species tested in this study.

Species	Origin	Specific Gravity	Extractive Content [%]	Wood Type
Tectona grandis	Myanmar, India	0.55-0.66	5.51	Hardwood
Dalbergia sissoo	Indian sub-continent	0.62-0.82	9.11	Hardwood
Morus alba	China	0.55-0.69	5.4	Hardwood
Cedrus deodara	Himalaya mountains	Un-known	9.67	Softwood
Pinus roxburghii	Pakistan, India, Nepal	0.43-0.49	7.4	Softwood

2.3 Termite Bioassay on Southern Yellow Pine Pressure Treated With Extractives.

Weighed and conditioned (33°C, 62 ± 3% R.H.) southern pine (SYP, *Pinus taeda* L sapwood blocks (19×19×19 mm) were pressure treated with different concentrations (2.5, 5 and 10 mg/ml) of each durable wood extractive. For control treatments, blocks were treated with solvent only (ethanol-toluene) or water. Blocks were pressure treated by placing five blocks in a 300 ml beaker containing the treatment solution in a vacuum-pressure chamber. Vacuum was turned on for 30 minutes and after that pressure was applied at 40 Psi for one hour. After pressure treatment, blocks were dried using paper towels, weighed, and conditioned again at 33°C and 62±3% R.H.

A termite bioassay was conducted according to a modified AWPA E1-15 (AWPA 2015). Modifications were the test block size of 19×19×19 mm and the amount of water added was 27ml. Screw top jars were filled with 150 grams sand along with 27 ml distilled water and left for two hours to equilibrate. After two hours, treated and control blocks were added to the jars so that each jar received only a single block. All treatments were replicated five times. A total of 400 termites (396 workers + 4 soldiers) were then added into each jar and jars were placed in a conditioning chamber at 27°C and 75±2 %R.H. for 28 days. After this period, the number of live termites were counted. Test blocks were brushed to remove sand and conditioned for one week in the conditioning chamber prior to being re-weighed to determine weight loss.

2.4 GC-MS Analysis of Extractives

Analyses were performed by coupling gas chromatography (GC) and mass spectrometry (MS) via an Agilent 7890B GC. An Agilent 19091S-433UI HP-5ms Ultra Inert column was used. The HP-5ms capillary column was of 0°C-325°C (350°C) 30m x 250 um x 0.25um. The temperature of the gas chromatography column was programed from 50-270°C. Solvent delay was set at 3-6 minutes. The temperature of ion source in the mass spectrometer was held at 230°C and the quad temperature was 150°C. For all extracts the sample size injected was 1µL. The Agilent 7890B GC was equipped with a split less injector at 270°C and an electron capture detector (µECD) at 250°C. Injection was done in the split less mode.

For extracts from *Tectona* grandis, the starting temperature was 75°C ramped to 230°C at 5°C/min and held for 80 minutes (Xie *et al.* 2011). For *Dalbergia sissoo* a dual ramp up was used where the starting temperature was 45°C ramped to 165°C at 4°C/min then ramped to 280°C at 4.5°C/min and held for 35 minutes (Aly *et al.* 2013). For *Morus* alba the starting temperature was 50°C ramped to 265°C at 5.5°C/min. and not held for any time (Sadeghifar *et al.* 2011, Se Golpayegani *et al.* 2014). For *Cedrus deodara* a dual ramp up was used where the starting temperature of 70°C ramped to 200°C at 10°C/min, held for 5 minutes then ramped to 300°C at 10°C/min and held for 10 minutes (Chaudhary *et al.* 2011b). For *Pinus roxburghii* a dual ramp up was used where the starting temperature of 45°C ramped to 165°C at 4°C/min then ramped to 280°C at 15°C/min and held for 9 minutes (Qadir and Shah 2014, Hassan and Amjid 2009). Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer scanned from m/z 3-700 at a rate of 2 scans per second. An integrator automatically calculated peak area. The top five compounds identified on a percentage of sample basis were identified using the NIST14 library.

2.5 Statistical Analysis

Percentage mortality data for exposure to treated SYP was subjected to a one way analysis of variance (ANOVA) using the MINITAB 17 program. Means were separated at the 5% significance level using Tukey's HSD test. Probit analysis also performed on the mortality data to calculate LC_{50} for each species treatment.

3. RESULTS AND DISCUSSION

3.1 Termiticidal effects of Southern Yellow Pine Pressure Treated with Extractives

Figure 1 shows the mortalities associated with each concentration of each species heartwood extractives. A linear dose response can be observed for all heartwood extracts tested, with the exception of *M. alba*, with increased concentration up to 10 mg/ml. Termites experienced 100% mortality at when exposed to SYP treated with 2.5, 5.0 and 10Mg/ml of *M. alba* extractives. Termites exposed to SYP treated with extracts of *Tectona grandis, Dalbergia sissoo, Morus alba*, and *Pinus roxburghii* experienced significantly higher mortality at the 10mg/ml treatment compared to the controls ($p<0.005$). Although the morality for termites exposed to SYP with extracts from *Cedrus deodara* was higher than the control mortalities, it was not significant at any of the levels tested compared to the control mortalities.

The results of Probit analysis gave the following LC_{50s}: *Tectona grandis* = 5.27mg/ml, *Dalbergia sissoo* = 3.27mg/ml, *Cedrus deodara* = 5.45mg/ml, *Morus alba* = 0.19 mg/ml, *Pinus roxburghii* = 2.5mg/ml. Since the highest mortalities were observed at the 10mg/ml concentration, we used this concentration for our GC-MS analysis to determine chemical components.

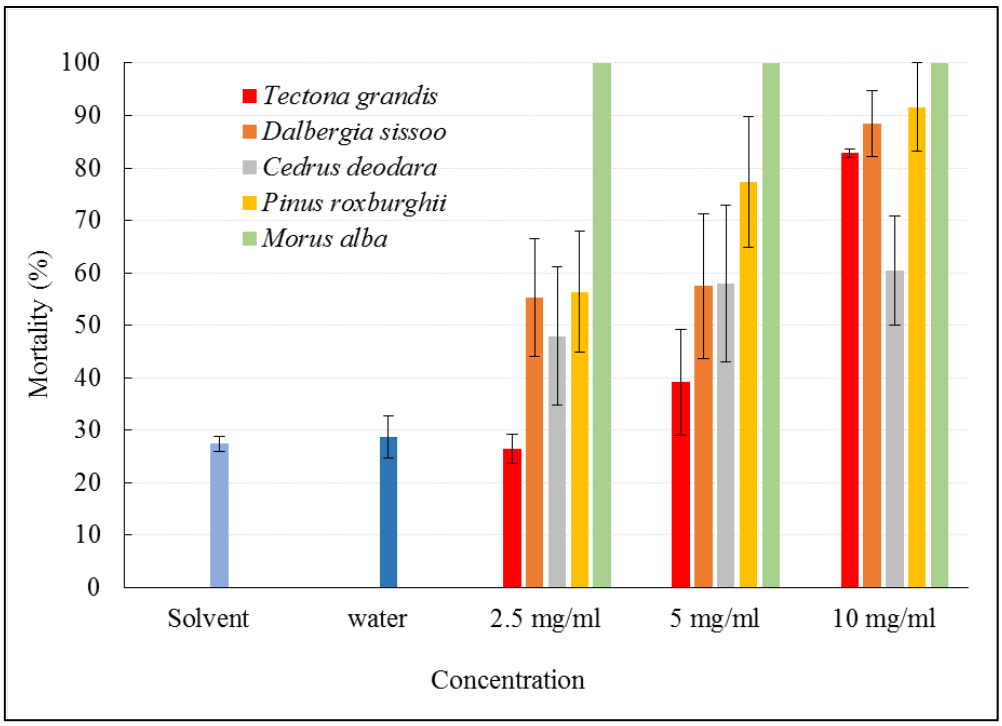

Figure 1: Effect of southern yellow pine treated with increasing concentrations of heartwood extractives on mortality of *R. flavipes* in a 28 day force feeding test.

3.2 GC-MS Analysis

3.2.1 Tectona grandis (Teak)

GC-MS analysis yielded 96 identified chemical compounds from *Tectona grandis* heartwood. Table 2 shows the retention time, molecular weights, identification quality and total percent of the compound in the sample, for the top five compounds identified from the solvent extract of this heartwood. These major compounds comprised approximately 64% of the total chemical makeup of the solvent fraction analyzed. The chromatogram associated with this analysis is shown in Figure 2. The top five compounds identified were 3-(1-Hydroxyethyl)-2-methyl-1-benzofuran-5,6-dicarbonitrile, 1-(1,1-dimethylethyl)-4-phenoxy-Benzene, 2-methyl-9,10-Anthracenedione, 1-Methyl-3,4-dihydroisoquinoline, and Squalene. Anthracenedione has also been identified in other studies as anthroquinoe and has been shown to have biocidal activity (Bhat *et al.* 2010, Gori *et al.* 2009, Lukmandaru and Ogiyama 2005, Lukmandaru *et al.* 2009, Xie *et al.* 2011). The compound squalene has also been shown to have biological activity (Kartal *et al.* 2006, Lukmandaru *et al.* 2009). Reyes-Chilpa, 1995 found that benzofurans had antitermitic properties. Anthracenedione (anthraquinone) and squalene made up the majority of the sample at 24.03 and 28.24 %, respectively.

Table 2: Five largest components from GC-MS analysis of solvent extracted *Tectona grandis*.

Compound Name	Retention Time [min]	Molecular Weight	Quality	% of Sample
3-(1-Hydroxyethyl)-2-methyl-1-benzofuran-5,6-dicarbonitrile	36.53	226.07	74.00	3.53
1-(1,1-dimethylethyl)-4-phenoxy-Benzene	39.98	226.14	72.00	3.06
2-methyl-9,10-Anthracenedione	42.18	222.07	97.00	24.03
1-Methyl-3,4-dihydroisoquinoline	56.67	264.12	45.00	5.22
Squalene	68.82	410.39	99.00	28.24

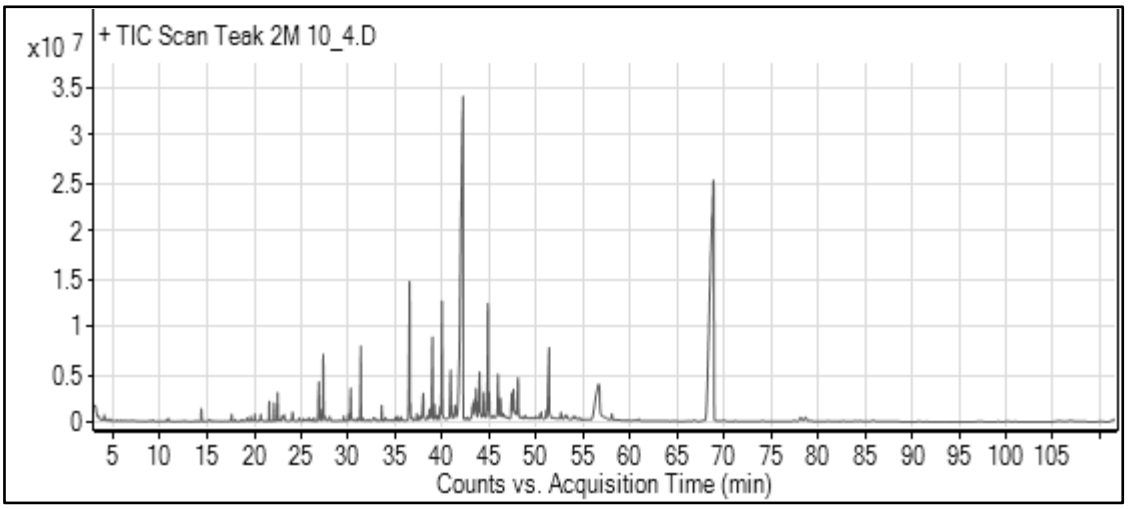

Figure 2: Chromatogram from GC-MS analysis of solvent extracted *Tectona grandis*

3.2.2 Dalbergia sissoo (Shisham)

GC-MS analysis yielded 52 identified compounds from *Dalbergia sissoo* heartwood. Table 3 shows the retention time, molecular weights, identification quality and total percent of the compound in the sample, for the top five compounds identified from the solvent extract of this heartwood. These major compounds comprised approximately 79% of the total chemical makeup of solvent fraction analyzed. The chromatogram associated with this analysis is shown in Figure 3. The top five compounds identified were 1, 2, 9-trimethoxy-Dibenzcycloheptane a cycloalkane, Trismethoxyresveratrol, 6, 8-dimethyl-Benzanthracene, 2-(3, 4-dimethoxyphenyl)-Indane-1, 3-dione, 1, 3-Diamino-8-n-butyl-5, 6-dihydrobenzoquinazoline. The most abundant compound was Trimethoxyresveratrol (40.6% of total). Trimethoxyresveratrol is a stilbene with published medicinal (Dias *et al*. 2013) and free radical scavenging activities (Shang *et al*. 2009). Stilbenes are regarded as potential sources of heartwood durability (Schultz and Nicholas 2000) and are present in many other examples of naturally durable woods (Hart and Shrimpton 1979). Resveratrol has been found to be biologically active and toxic to insects in previous studies (Aly *et al.* 2013, Hart 1981, Hart and Shrimpton 1979, Harmatha and Dinan 2003, Nascimento *et al.* 2013).

Table 3: Five largest components from GC-MS analysis of solvent extracted *Dalbergia sissoo*.

Compound Name	Retention Time [min]	Molecular Weight	Quality	% of Sample
1,2,9-trimethoxy-Dibenzcycloheptane	42.33	284.141	70	3.09
Trismethoxyresveratrol	44.49	270.126	64	40.6
6,8-dimethyl-Benzanthracene	45.44	256.125	64	11.41
2-(3,4-dimethoxyphenyl)-Indane-1,3-dione	57.58	282.089	58	7.25
1,3-Diamino-8-n-butyl-5,6-dihydrobenzoquinazoline	60.03	268.146	59	17.09

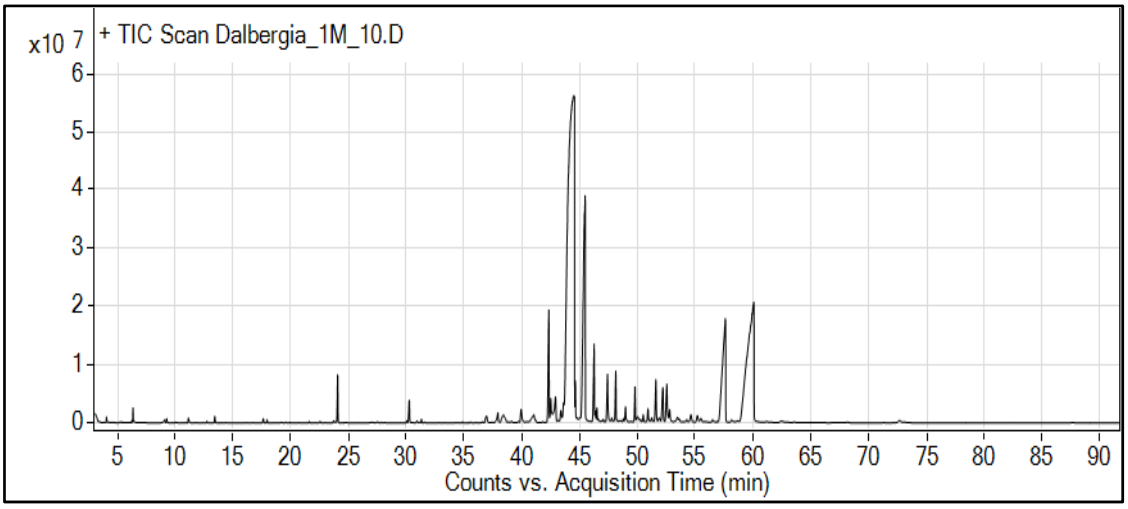

Figure 3: Chromatogram from GC-MS analysis of solvent extracted *Dalbergia sissoo*.

3.2.3 Morus alba (White Mulberry)

GC-MS analysis yielded 55 identified compounds from *Morus alba* heartwood. Table 4 shows the retention time, molecular weights, identification quality and total percent of the compound in the sample, for the top five compounds identified from the solvent extract of this heartwood. These major compounds comprised approximately 58% of the total chemical makeup of the solvent fraction analyzed. The chromatogram associated with this analysis is shown in Figure 4. The top five compounds identified were Resorcinol, 2, 4-dihydroxy-Benzaldehyde, gamma-Sitosterol (Kirker *et al.*, 2013), 4, 14-dimethyl-9, 19-Cycloergost-24(28)-en-3-ol (Hosseinihashemi and Kanani 2012), 24-methylene-9, 19-Cyclolanostan-3-ol. Resorcinol was the main component identified and consisted of 40.54 % of the sample. Resorcinol has been identified from *Morus alba* in high quantities in other studies that also found it showed biological activity (Se Golpayegani *et al.* 2014, Salem *et al.* 2013, Sadeghifar *et al.* 2011). Synthetic resorcinol has been studied as an antitermitic compound for protection of wood (Lyons 1936). Resorcinol has been shown to have antifungal properties and insecticidal anti-termitic properties against *Coptotermes formosanus* (Salem *et al.* 2013, Mansour *et al.* 2015) The results of this study strongly indicate that extractives from *Morus alba* heartwood had significant negative impact on the termite activity.

Table 4: Five largest components from GC-MS analysis of solvent extracted *Morus alba*.

Compound Name	Retention Time [min]	Molecular Weight	Quality	% of Sample
Resorcinol	7.78	110.037	94	40.54
2,4-dihydroxy-Benzaldehyde	9.71	138.03	95	6.97
gamma-Sitosterol	26.81	414.39	99	4.31
4,14-dimethyl-9,19-Cycloergost-24(28)-en-3-ol	27.27	426.39	78	3.68
24-methylene-9,19-Cyclolanostan-3-ol	28.62	440.4	93	2.85

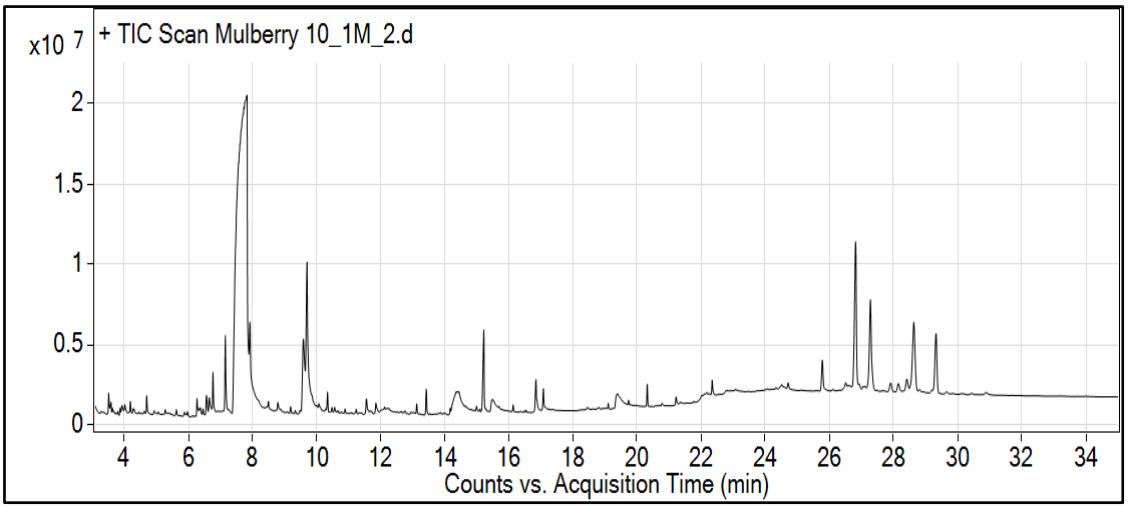

Figure 4: Chromatogram from GC-MS analysis of solvent extracted *Morus alba*.

3.2.4 Cedrus deodara (Cedar)

GC-MS analysis yielded 147 identified compounds from *Cedrus deodara* heartwood. Table 5 shows the retention time, molecular weights, identification quality and total percent of the compound in the sample, for the top five compounds identified from the solvent extract of Cedar heartwood. These major compounds comprised approximately 37% of the total chemical makeup of the solvent fraction analyzed. The chromatogram associated with this analysis is shown in Figure 5. The top five compounds identified were sesquiterpenes alpha-Cuprenene, beta-Himachalene oxide, Di-epi-alpha-cedrene, (Z)-alpha-Atlantone, and (E)-Atlantone. Forms of Atlantone compromised 21.08 % of the sample. This sesquiterpene has been shown to have biological activity (Bacci *et al.* 2015, Chaudhary *et al.* 2011b). Cuprenene, Himachalene and Cedrene have been found associated with frontal gland secretions of soldier termites and are secreted as repellents (Singh and Agarwal 1988, Khan and Naheed 1990, Quintana *et al.* 2003, Piskorski *et al.* 2009).

The five largest components from *Cedrus deodara* compromised a much smaller amount of the total compared to the other extractives analyzed. This species also yielded the largest number of identified peaks at 147. This in part could explain the lower toxicity of this species to *R. flavipes* compared to the other heartwood species tested in the feeding test.

Table 5: Five largest components from GC-MS analysis of solvent extracted *Cedrus deodara*.

Compound Name	Retention Time [min]	Molecular Weight	Quality	% of Sample
alpha-Cuprenene	18.785	204.188	99	3.84
beta-Himachalene oxide	22.819	220.183	99	3.62
Di-epi-alpha-cedrene	24.197	204.188	90	8.14
(Z)-alpha-Atlantone	26.545	218.167	99	3.16
(E)-Atlantone	28.893	218.167	99	17.92

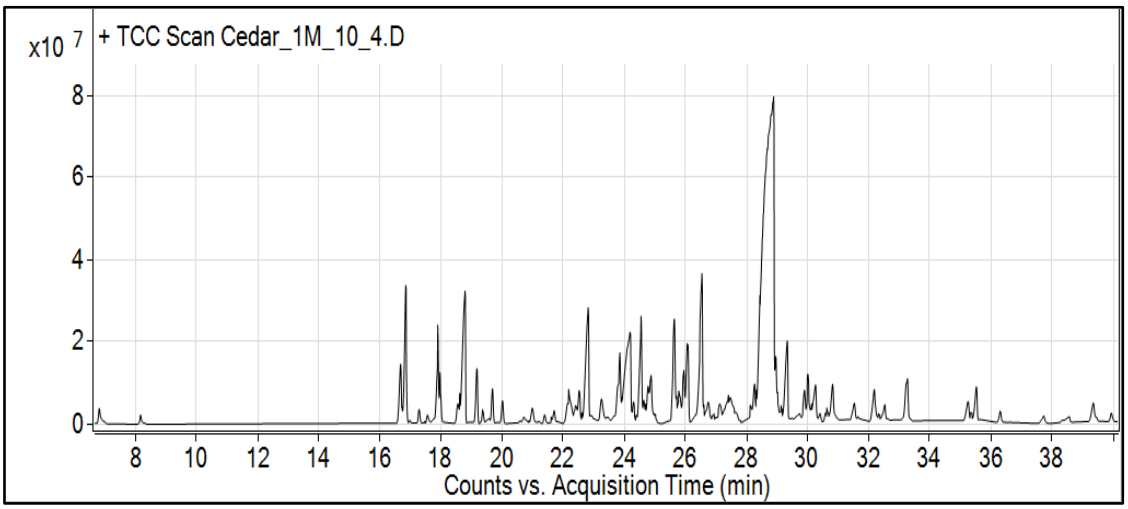

Figure 5: Chromatogram from GC-MS analysis of solvent extracted *Cedrus deodara*.

3.2.5 Pinus roxburghii (Chir Pine)

GC-MS analysis yielded 12 identified compounds from *Pinus roxburghii* heartwood. Table 6 shows the retention time, molecular weight, identification quality and total percent of the compound in the sample, for the top five compounds identified from the solvent extract of its heartwood. These major compounds comprised approximately 87% of the total chemical makeup of the solvent fraction analyzed. The chromatogram associated with this analysis is shown in Figure 6. The top five compounds identified were 1,2,3,4-tetrahydro-5,8-dimethyl-Acridin-9-amine, 2,3-dihydro-5-hydroxy-7-methoxy-2-phenyl-4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-Benzopyran-4-one,-5-hydroxy-7-methoxy-2-phenyl-4H-1-Benzopyran-4-one, and chrysin. Forms of benzopryran compromised 51% of the sample. Duchowicz *et al.* (2009) examined the toxicity of benzopyran. Acridin-9-amine was also identified as 27.46 % of the sample. This compound was not found in the literature to be toxic or biologically active and we are not certain of its function. Morimoto *et al.* (2003) found chrysin to be a feeding stimulant in insects. Ohmura *et al.* (2000) found anti-feeding in *Coptotermes* caused by flavonoids from cedar oil.

Table 6: Five largest components from GC-MS analysis of solvent extracted *Pinus roxburghii*.

Compound Name	Retention Time [min]	Molecular Weight	Quality	%of Sample
1,2,3,4-tetrahydro-5,8-dimethyl-Acridin-9-amine	37.61	226.147	70	27.46
2,3-dihydro-5-hydroxy-7-methoxy-2-phenyl-4H-1-Benzopyran-4-one	38.21	270.089	99	9.18
2,3-dihydro-5,7-dihydroxy-2-phenyl-4H-1-Benzopyran-4-one	38.69	256.074	99	24.42
5-hydroxy-7-methoxy-2-phenyl-4H-1-Benzopyran-4-one	39.426	268.074	99	17.41
Chrysin	40.01	254.058	96	8.62

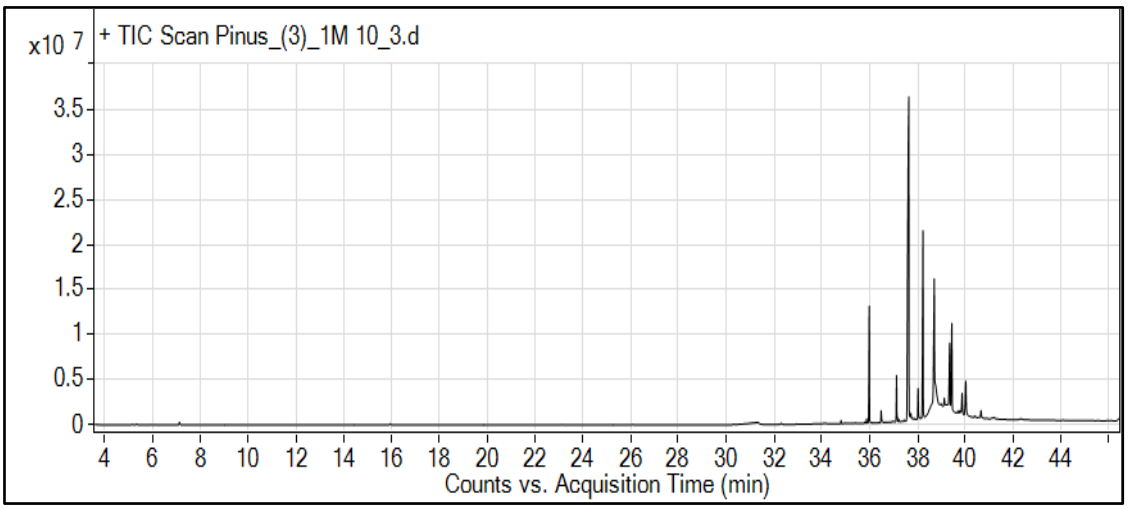

Figure 6: Chromatogram from GC-MS analysis of solvent extracted *Pinus roxburghii*.

4. CONCLUSIONS

Extracts of *Tectona grandis, Dalbergia sissoo, Cedrus deodara, Morus alba, Pinus roxburghii,* showed toxicity to *Reticulitermes flavipes* when applied to non-durable pine. Extracts from *Morus alba* in particular were found to be highly toxic to *R. flavipes* and this compound is being examined further. GC-MS Analysis of solvent extracts generally showed 2-3 compounds made up much of the sample and that previous literature suggested those compounds were likely the main factors in the increased termite mortality observed as they had been noted to be fungicidal or insecticidal. Compounds identified from *Cedrus deodara* in particular have been found to be associated with defensive, repellent chemicals used by soldier termites (Singh and Agarwal 1988, Khan and Naheed 1990, Quintana *et al.* 2003, Piskorski *et al.* 2009).

Future work will focus on more accurate compound identification via GC-MS analysis and testing of isolated toxic components identified. These toxic components will be examined for synergies with combinations of other botanical or less toxic biocides.

5. ACKNOWLEDGEMENTS

The authors wish to thank Craig Bell, USFS FPL Starkville, for wood block and shaving preparation and Dr. Hamid Borazjani, Mississippi State University, for use of his laboratory facilities. The authors also gratefully acknowledge the financial support of the Higher Education

Commission of Pakistan (HEC) and the USDA-FS International Program who granted a fellowship to Babar Hassan to conduct research at the USDA-FS Forest Products Laboratory.

6. REFERENCES

Aly, H M, El-Sayed, A B, Gohar, Y M, Salem, M M (2013): The Value-Added Uses of *Ficus Retusa* and *Dalbergia sissoo* Grown in Egypt: GC/MS Analysis of Extracts. *Journal of Forest Products and Industries*, 2(3), 34-41.

American Wood Protection Association (2015): AWPA E1-15 Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites. *AWPA Book of Standards* pp. 387-395. American Wood Protection Association. Birmingham, Alabama.

Arango, A R, Green III, F, Hintz, K, Lebow, P K, Miller, R B (2006): Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). *International Biodeterioration and Biodegradation*. 57: 146-150.

ASTM D1105-96. Standard Test Method for Preparation of Extractive-Free Wood. 2014. ASTM- International, & American Society for Testing & Materials. *Annual book of ASTM standards*. American Society for Testing & Materials. West Conshocken, PA. Vol. 4.10, pp. 430-432.

Bacci, L, Lima, J K, Araújo, A A, Blank, A F, Silva, I, Santos, A A, Santos, A C, Alves, P B, Picanço, M C, (2015): Toxicity, behavior impairment, and repellence of essential oils from pepper-rosmarin and patchouli to termites. *Entomologia Experimentalis et Applicata*, *156*(1), pp.66-76.

Bhat, K M, Florence, E M (2003): Natural decay resistance of juvenile teak wood grown in high input plantations. *Holzforschung*, 57(5), 453-455.

Bhat, I H, Khalil, A S, Shuib, N S, Noor, A M (2010): Antifungal activity of the heartwood extracts and their constituents from cultivated *Tectona grandis* against *Phanerochaete chrysosporium*. *Wood Research*, *55*, pp.59-66.

Burgess, K S, Husband, B C (2006): Habitat differentiation and the ecological costs of hybridization: the effects of introduced mulberry (*Morus alba*) on a native congener (*M. rubra*). *Journal of Ecology*, 94(6), 1061-1069.

Chaudhary, A K, Ahmad, S, Mazumder, A (2011a): *Cedrus deodara* (Roxb.) Loud. A review on its ethnobotany, phytochemical and pharmacological profile. *Pharmacognosy Journal*, 3(23), 12-17.

Chaudhary, A, Sharma, P, Nadda, G, Kumar, T D, Bikram, S (2011b): Chemical composition and larvicidal activities of the Himalayan cedar, *Cedrus deodara* essential oil and its fractions against the diamondback moth, *Plutella xylostella*. *Journal of Insect Science*, *11*(1), p.157.

Da Costa, E W B, Rudman, P, Gay, F J (1958): Investigations on the durability of *Tectona grandis*. *Empire Forestry Review*, 291-298.

Dias, S J, Li, K, Rimando, A M, Dhar, S, Mizuno, C S, Penman, A D,Levenson, A S (2013): Trimethoxy-Resveratrol and Piceatannol Administered Orally Suppress and Inhibit Tumor Formation and Growth in Prostate Cancer Xenografts. *The Prostate*, 73(11), 1135-1146.

Duchowicz, P R, Goodarzi, M, Ocsachoque, M A, Romanelli, G P, Ortiz, E V, Autino, J C, Bennardi, D O, Ruiz, D M, Castro, EA (2009): QSAR analysis on *Spodoptera litura* antifeedant activities for flavone derivatives. *Science of the total environment*, *408*(2), pp.277-285.

Gori, G, Carrieri, M, Scapellato, M L, Parvoli, G, Ferrara, D, Rella, R, Bartolucci, G B (2009): 2-methylanthraquinone as a marker of occupational exposure to teak wood dust in boatyards. *Annals of occupational hygiene*, *53*(1), 27-32.

Harmatha, J and Dinan, L (2003): Biological activities of lignans and stilbenoids associated with plant-insect chemical interactions *Phytochemistry Reviews* 2: 321–330.

Hart, J H (1981): Role of phytostilbenes in decay and disease resistance. *Annual review of Phytopathology*, *19*(1), pp.437-458.

Hart, J H, Shrimpton, D M (1979): Role of stilbenes in resistance of wood to decay. *Phytopathology*, 69(10), 1138-1143.

Hassan, A and Amjid, I (2009): Gas chromatography-mass spectrometric studies of essential oil of Pinus roxburghaii stems and their antibacterial and antifungal activities. *Journal of Medicinal Plants Research*, *3*(9), pp.670-673.

Haupt, M, Leithoff, H, Meier, D, Puls, J, Richter, H G, Faix, O (2003): Heartwood extractives and natural durability of plantation-grown teakwood (*Tectona grandis* L.)—a case study. *European Journal of Wood and Wood Products*, 61(6), 473-474.

Hosseinihashemi, S K and Kanani, S (2012): Heartwood extractives of *Robinia pseudoacacia* wood. *Journal of Advanced Laboratory. Researchin Biology*, *3*(2), pp.131-134.

Imamura, H (1989): Contribution of extractives to wood characteristics. *In Natural Products of Woody Plants* (pp. 843-860). Springer Berlin Heidelberg.

Kartal, S M, Hwang, W J, Imamura, Y, Sekine, Y (2006): Effect of essential oil compounds and plant extracts on decay and termite resistance of wood. *Holz als Roh- und Werkstoff* (2006) 64: 455–461.

Khan, N, Naheed, S (1990): Chemical Investigation of *Cedrus Deodara* Stem-Bark ll. Isolation and Identification of Some Sesquiterpene Hydrocarbons. *Journal of Chemical Society of Pakistan. Vol*, *12*(0), p.4.

Khan, M M, Khan, M H, Appanah, S, Allard, G, Amatya, S M (2000): Die-back of *Dalbergia sissoo* in Pakistan. *Field Document-FORSPA*, (18), 51-56.

Kirker, G T, Clausen, C A, Blodgett A B, Lebow, S T (2013): Evaluating naturally durable wood species for repair and rehabilitation of above-ground components of covered bridges. *General Technical Report 224 FPL*. 1-43.

Lukmandaru, G, Takahashi, K (2009): Radial distribution of quinones in plantation teak (*Tectona grandis* Lf). *Annals of Forest Science,* 66(6), 1-9.

Lukmandaru, G and Ogiyama, K (2005): Bioactive compounds from ethyl acetate extract of teakwood (*Tectona grandis* Lf). In *Proceedings of the 6th International Wood Science Symposium LIPI-JSPS Core, Bali, Indonesia* (pp. 346-350).

Lukmandaru, G, Ashitani, T, Takahashi, K (2009): Colour and chemical characterization of partially black-streaked heart-wood in teak (*Tectona grandis*). *Journal of Forestry Research*, *20*(4), pp.377-380.

Lyons, F H (1936): Impregnation composition for wood and the like. *US. Patent 2,041,647*.

Mansour, M A, Salem, M M, Khamis, M H (2015): Natural durability of C*itharexylum spinosum* and Morus alba woods against three mold fungi. *Bioresource Technology*. 10: 5330–5344.

Manzoor, F, Mir, N (2010): Survey of termite infested houses, indigenous building materials and construction techniques in Pakistan. *Pakistan Journal of Zoology*, 42(6), 693-696.

Morimoto, M, Tanimoto, K, Nakano, S, Ozaki, T, Nakano, A, Komai, K (2003): Insect antifeedant activity of flavones and chromones against *Spodoptera litura*. *Journal of agricultural and food chemistry*, *51*(2), pp.389-393.

Morris, P, Laks, P, Lebow, S (2011): Standardization of naturally durable wood species. *Proceeding of the American Wood Protection Association*. American Wood Protection Association, Birmingham, AL.

Nascimento, M S, Santana, A D, Maranhão, C. A, Oliveira L S, Bieber, L (2013): Phenolic Extractives and Natural Resistance of Wood. *Intech* 13 (2013): 349-370.

Ohmura, W, Doi, S, Aoyama, M, Ohara, S (2000): Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of wood Science*, *46*(2), pp.149-153.

Omidiran, M O, Baiyewu, R A, Ademola, I T, Fakorede, O C, Toyinbo, E O, Adewumi, O J, Adekunle, E A (2012): Phytochemical analysis, nutritional composition and antimicrobial activities of white mulberry (*Morus alba*). *Pakistan Journal of Nutrition*, 11(5), 456.

Ordonez, A L, Gomez, J D, Vattuone, M A, Isla, M I (2006): Antioxidant activities of *Sechlumedule* (Jacq.) swart extracts. *Food Chemistry*, 97, 452-458.

Pandey, D, Brown, C (2000): Teak: a global overview. *UNASYLVA-FAO*-, 3-13.

Piskorski, R, Hanus, R, Kalinova, B, Valterova, I, KŘeČek, J, Bourguignon, T, Roisin, Y, Šobotník, J (2009): Temporal and geographic variations in the morphology and chemical composition of the frontal gland in imagoes of Prorhinotermes species (Isoptera: Rhinotermitidae). *Biological Journal of the Linnean Society*, *98*(2), pp.384-392.

Qadir, M, Shah, W A (2014): GC-MS analysis, antibacterial, antioxidant and anticancer activity of essential oil of *Pinus roxburghii* from Kashmir, India. *International Journal of Researchin Pharmacy and Chemistry*, *4*(1), pp.228-232.

Quintana, A, Reinhard, J, Faure, R, Uva, P, Bagnères, A G, Massiot, G, Clément, J L (2003): Interspecific variation in terpenoid composition of defensive secretions of European Reticulitermes termites. *Journal of Chemical Ecology*, *29*(3), pp.639-652.

Qureshi, H, Arshad, M, Bibi, Y (2014): Invasive flora of Pakistan: a critical analysis. *Journal of Biodiversity and Environmental Science*, 4, 407-424.

Reyes-Chilpa, R, Viveros-Rodriguez, N, Gomez-Garibay, F, Alavez-Solano, D (1995): Antitermitic activity of *Lonchocarpus castilloi* flavonoids and heartwood extracts. *Journal of Chemical Ecology*, *21*(4), pp.455-463.

Rowe, J W, and Conner, A H (1979): Extractives in eastern hardwoods: a review. *General technical report/Forest products laboratory*. Forest service. USDA (No. FPL 18).

Sadeghifar, H, Sheikh, L I, Khalilzadeh, M I, Ebadi, A G (2011): Heartwood Extractives of Iranian *Morus alba* Wood. *Jornal of Chemical. Society of Pakistan*. 33(1): 104-106.

Salem, M M, Aly, H M, Gohar, Y M, EL-Sayed, A B (2013): Biological activity of extracts from *Morus alba* (L.), *Albizzia lebbeck* (L.) Benth, and *Casuarina glauca* Sieber against the growth of some pathogenic bacteria. *International Journal of Agriculture and Food Research.* 2(1), 9-22.

Scheffer, T C (1966): Natural resistance of wood to microbial deterioration. *Annual Review of Phytopathology*, *4*(1), 147-168.

Scheffer, T C, Morrell, J J (1998): Natural durability of wood: a worldwide checklist of species. Corvallis, OR, College of Forestry, Forest Research Laboratory, Oregon State University.

Schultz, T P, Nicholas, D D (2000): Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. *Phytochemistry*, 54(1), 47-52.

Se Golpayegani, A, Thevenon, M F, Gril, J, Masson, E, Pourtahmasi, K (2014): Toxicity potential in the extraneous compounds of white mulberry wood (*Morus alba*). Maderas. *Cienc. y Tecnol.* 16(2): 227-238.

Seshadri, T R (1972): Polyphenols of Pterocarpus and Dalbergia woods. *Phytochemistry*, 11(3), 881-898.

Shang, Y J, Qian, Y P, Liu, X D, Dai, F, Shang, X L, Jia, W Q, Zhou, B (2009): Radical-scavenging activity and mechanism of resveratrol-oriented analogues: influence of the solvent, radical, and substitution. *The Journal of Organic Chemistry*, 74(14), 5025-5031.

Sheikh, M I (1989): *Dalbergia sissoo*, Roxb. Its production, management and utilization in Pakistan. Regional Wood Energy Development Programme in Asia (GCP/RAS/111/NET) *Field Document 21*. Bangkok.

Shuaib, M, Ali, M, Ahamad, J, Naquvi, K J, Ahmad, M I (2013): Pharmacognosy of *Pinus roxburghii:* a Review. *Journal of Pharmacognosy and Phytochemistry*, 2(1).

Singh, D and Agarwal, S K (1988): Himachalol andβ-himachalene: Insecticidal principles of Himalayan cedarwood oil. *Journal of Chemical Ecology, 14*(4), pp.1145-1151.

Taylor, A M, Gartner, B L, Morrell, J J (2002): Heartwood formation and natural durability: A review. *Wood and Fibre Science*, 34(4), 587-611.

Tewari, D N (1994a): A monograph on *Dalbergia sissoo* Roxb. *International Book Distributors*.

Tewari, D N (1994b): A monograph on Chir Pine (*Pinus roxburghii* Sarg.). *International Book Distributors.*

Xie, C P, Li, K F, Lin, J L, Li, J B (2011): GC-MS Analysis on heartwood extractive chemical components of different provenances teak (*Tectona grandis* Lf). In *Advanced Materials Research*, 236, 1049-1053).