EFFECT OF WALNUT HEARTWOOD EXTRACTIVES, ACID COPPER CHROMATE, AND BORIC ACID ON WHITE-ROT DECAY RESISTANCE OF TREATED BEECH SAPWOOD

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This study evaluates the individual and interaction effects of wood extractives, acid copper chromate (ACC), and boric acid (B) on the resistance to fungus of treated wood species. Walnut (*Juglans regia* L.) heartwood extractives were extracted with hot water, methanol, and ethanol solvents. Test specimens were prepared from beech sapwood (*Fagus orientalis*) to meet BS 838 (1961) requirements, then exposed to white-rot fungus, *Trametes versicolor*, for 14 weeks under laboratory conditions. Extractives of walnut heartwood contributed to increased resistance against fungus attack in the presence of B preservative only. The lowest weight loss (0.12%) occurred in the samples treated with 3.5% hot water extract and 1% boric acid, and the highest weight loss occurred in the control samples (23.7%). Results indicated that there was significant difference between the weight loss and actual retention for all treatments, but there was not any significant difference between the weight loss of treatments containing B preservative. The weight loss of samples treated with hot water extract (18.32%) was less than samples treated with methanol and ethanol extracts (21.5% and 23.1%, respectively). There was significant difference between the individual and interaction effects of wood extractives on the resistance to fungus of treated wood species. An emulsified mixture of B and walnut heartwood extractives controlled decay fungus on beech wood better than the mixture of ACC and walnut heartwood extractives, but ACC alone controlled decay fungus on beech wood better than the emulsified mixture of ACC and walnut heartwood extractives.

Keywords: Heartwood; Extractives; Acid copper chromate; Boric acid; Decay resistant; Trametes versicolor

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INTRODUCTION

Wood is a natural and renewable resource often used as construction material in decks, fences, utility poles, *etc*. However, if sufficient moisture is present, lumber, pole, and wood composites can be attacked and deteriorated through the action of a wide variety of organisms, such as fungi, insects, termites, and marine boring animals (Green III and Schultz 2003; Leightley 2003). This deterioration can be prevented if certain construction practices are followed, which create unsuitable conditions for the activities of these destructive factors, such as keeping wood dry. If the wood product cannot be kept dry or is in ground contact or an aquatic environment, then wood must be protected using biocide treatments (Green III and Schultz 2003; Leightley 2003).

Traditional wood preservatives generally provide a broad spectrum of activity against wood-degrading organisms, but they are regulated or restricted owing to environmental concerns over the toxic compounds in their formulation. Environmental hazards and the costs of certain wood preservatives result in minimization of the amount of active compounds in wood preservative systems and an increase in the need for the development of alternative, more environmentally benign wood preservatives (Schultz and Nicholas 2000; Leightley 2003). Novel wood protection formulations, based upon mixtures of already developed (non-metallic or organic) biocides, offer some real advantages to the wood protection industry. One potential approach to developing new wood preservatives is to use heartwood or heartwood extractives, because the compounds in heartwood possess both fungicidal activity and excellent free radical scavengers (Schultz and Nicholas 2000). Heartwood extractives can provide synergistic effects with wood preservatives for protection against biodegradation on account of its natural durability (Hwang *et al.* 2006). Certain wood species may have specific components in their heartwood portions that are effective against a number of organisms (Taylor *et al.* 2002; Schultz and Nicholas 2002).

The combination of one or more organic biocide (s) with various non-biocidal additives might be one method to reduce the cost. An alternative and potentially elegant method would be to identify an additive that would disrupt the initial generation of the pre-radical oxidant in the acidic region of the fungal mycelium, and thus prevent the wood cell wall from being perturbed while possibly also causing the oxidant to form a radical near the fungus. Last but not least, metals such as Fe or Mn are well known to be involved in fungal degradation mechanisms, either as part of an enzymatic system or as a free metal. Thus, the addition of appropriate metal chelators might prevent the metals from being available to the fungi (Suttie 1997; Goodell *et al.* 1997; Schultz and Nicholas 2002). Other mechanisms, essential biocides, and chelating metals, might also explain the enhanced efficacy obtained when a biocide and metal chelators are combined (Green III and Schultz 2003). The heartwoods of naturally decay-resistant woods are usually resistant to termites. Thus, the combination of biocides, antioxidants, and/or metal chelators may be helpful in protecting wood against both termites and fungi (Green III and Schultz 2003). The fungicidal properties of a vast majority of extractives have been found to be mediocre at best when compared to commercial biocides (Schultz *et al.* 1995). However, the various phenolic extractives are known to be excellent antioxidants (Larson 1988; Hadi *et al.* 2000; Cooper-Drive and Bahattacharya 1998), and many of these phenolics also have metal-chelating properties (Cooper-Drive and Bahattacharya 1998). Thus, the combination of a biocide, antioxidant, and/or metal chelator might simply mimic nature's approach to make wood durable. For example, the combination of various antioxidants and/or metal chelators increase the efficacy of a wide variety of organic biocides (Schultz and Nicholas 2001, 2002). Furthermore, gallic acid derivatives (Kishino *et al.* 1995), derived from the tannic acids, can be found in any walnut heartwood (Hosseini Hashemi and Jahan Latibari 2011). It can enhance the efficacy of the relatively expensive biocide propiconazole (Schultz and Nicholas 2002).

According to Su *et al.* (2007), the heartwood and bark of *Maackia amurensis* Ruper.et.Maxim. were used as raw material to obtain 10 kinds of extracts by extracting with ether, chloroform, acetone, methanol, and water, respectively, and antibacterial

activities against *Coriolus versicolor* and *Gloeophyllum trabeum* of 10 kinds of extracts were studied. It was found that methanol and acetone extracts of heartwood have good antibacterial activities. Wood preservation activities of methanol extract of heartwood was also compared with the wood preservative acid copper chromate (ACC) in the lab. Weight loss and scanning electron microscopy (SEM) photography showed that methanol extract of heartwood has better decay resistance against *C. versicolor* and weaker decay resistance against *G. trabeum*, but ACC has better decay resistance against two epiphytes. The acute toxicities of methanol extract of heartwood and ACC to *Brachydanio rerio* were studied. At 24, 48, and 72 h, LC50 of methanol extracts were 16.5, 12.7, and 12.0 mg/L, respectively, and the LC50 values of ACC were 3.2, 2.5, and 2.0 mg/L respectively. The safe concentration of methanol extract and ACC are 2.26 and 0.46 mg/L, respectively.

Borates are used in large quantities in building products in Asia and North America, in wood composites and pest control in North America, and in formulation of exterior and remedial treatments in Europe (Lloyd 1997). Because boric acid and borates are water-soluble, their use in outdoor wood products is limited. Research to find more leach-resistant borates for wood products has been performed in recent years. Chromate copper borates (Ochrymowych and McOrmond 1978) performed well in field tests; however, chromium may not be used in products because of environmental concerns. The effectiveness of copper borates was attributed to the permanence of copper in wood.

The objective of the present work was to evaluate the individual and interaction effects of walnut heartwood extractives, B, and ACC preservatives to protect non-durable beech wood against fungal attack.

EXPERIMENTAL

Preparation of Test Specimens

Defect-free beech (*Fagus orientalis*) wood was first cut into 50 x 25 x 15 mm^3 (L x T x R) blocks (Fig. 1). All specimens were oven-dried at 103 ± 2 ºC for 48 h before and after treatment.

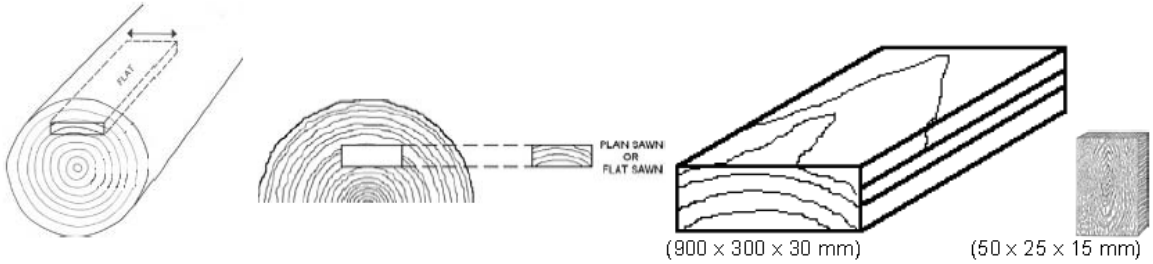


Figure 1. Specimens preparation from the log

Preparation of Treatment Solutions

Extraction process and preservative treatment of wood specimens

Walnut heartwood was ground into small pieces and sieved between 40 and 60 mesh screens. Walnut heartwood powder was extracted according to the TAPPI method 207 om-93 in a 1000 mL Erlenmeyer flask with hot water at 85 ± 5 ºC called HWE. Also,

walnut heartwood powder was soxhlet extracted according to the TAPPI method 207 om-77 with methanol and ethanol solvents for 6 to 8 h until a colorless solution was obtained. The names and concentrations of the solution (s) and extract (s) used, were prepared as in Table 1.

Table 1. Chemicals Used in the Treatments and their pH

Extract Extract:Salt (s)	pH	Extract Extract:Salt (s)	pH	Extract Extract:Salt (s)	pH
2% ACC	4.22	1% B	5.43	2% ACC:1% B	4.45
3.5% HWE	4.79	3.5% MeE	5.85	3.5% EtE	5.14
3.5% HWE:2% ACC	5.17	3.5% MeE:2% ACC	5.29	3.5% EtE:2% ACC	5.13
3.5% HWE:1% B	4.65	3.5% MeE:1% B	4.35	3.5% EtE:1% B	4.29
3.5% HWE:2% ACC:1% B	5.47	3.5% MeE:2% ACC:1% B	5.20	3.5% EtE:2% ACC:1% B	5.32

HWE = Hot water extract; MeE = Methanol extract; EtE = Ethanol extract; B = Boric acid; ACC = Acid-Copper-Chromate

In total, we examined 15 different chemical treatments with one untreated control treatment. Four replicates for each treatment were employed.

Treatment Method

Wood samples were treated using a long-term dipping process at atmospheric pressure for 25 days to reach complete saturation. Treated blocks were then air-dried for 20 days to ensure fixation of the chemicals within the test specimens. Untreated blocks were used as controls. After the impregnation, wood samples were removed from the treatment solution, wiped gently to remove excess solution from the wood surface, and weighed (to the nearest 0.01g) to determine the actual retention,

$$\text{Actual Retention} = (M_b - M_a)/V \quad (1)$$

where M_a and M_b (*kgs*) denote the oven-dry weights of wood prior to and after impregnation and V (*cubic meter*) denote the oven-dry volume of wood after impregnation with different chemical treatments (Fig. 2a, b, c, and d).

Wood Decay Test

Four treated specimens from each treatment and their respective untreated controls were exposed to fungal attack. Beech wood was exposed in Kolle flasks containing agar medium inoculated with a white-rot fungus *Trametes versicolor*, according to BS 838 (1961). All specimens were incubated under controlled conditions (75% relative humidity and 25 ºC) for 14 weeks then brushed and dried at 103 ± 2 ºC.

Evaluation of fungal performance was carried out by visual inspection and mass loss. Fungal mycelium growth was measured according to the Willeitner scale (1984).

Statistical Analysis

Actual retention and weight loss values were evaluated using a computerized SPSS 17.0 statistical program and tested with ANOVA, followed by a Duncan's multiple range test (DMRT) with 95% confidence levels.

RESULTS AND DISCUSSION

Amount of Actual Retention of Wood Preservatives

The actual retention of different treating solutions in the treated wood specimens are shown in Fig. 2 a, b, c, and d.

The Effects of Extractives and Wood Preservatives on the Decay Resistance of Beech Treated Wood

The individual and the interaction effects of walnut heartwood extractives, ACC, and B treatments to a white-rot fungus were studied to determine whether there were interactions and enhanced actions against the fungus (Fig. 2a, b, c, and d).

The variation in fungal mycelium growth and weight loss of the specimens against the white-rot fungus (*Trametes versicolor*) for 14 weeks were measured. The results show that depending on the treating solutions, there were variations in fungal mycelium growth and weight loss.

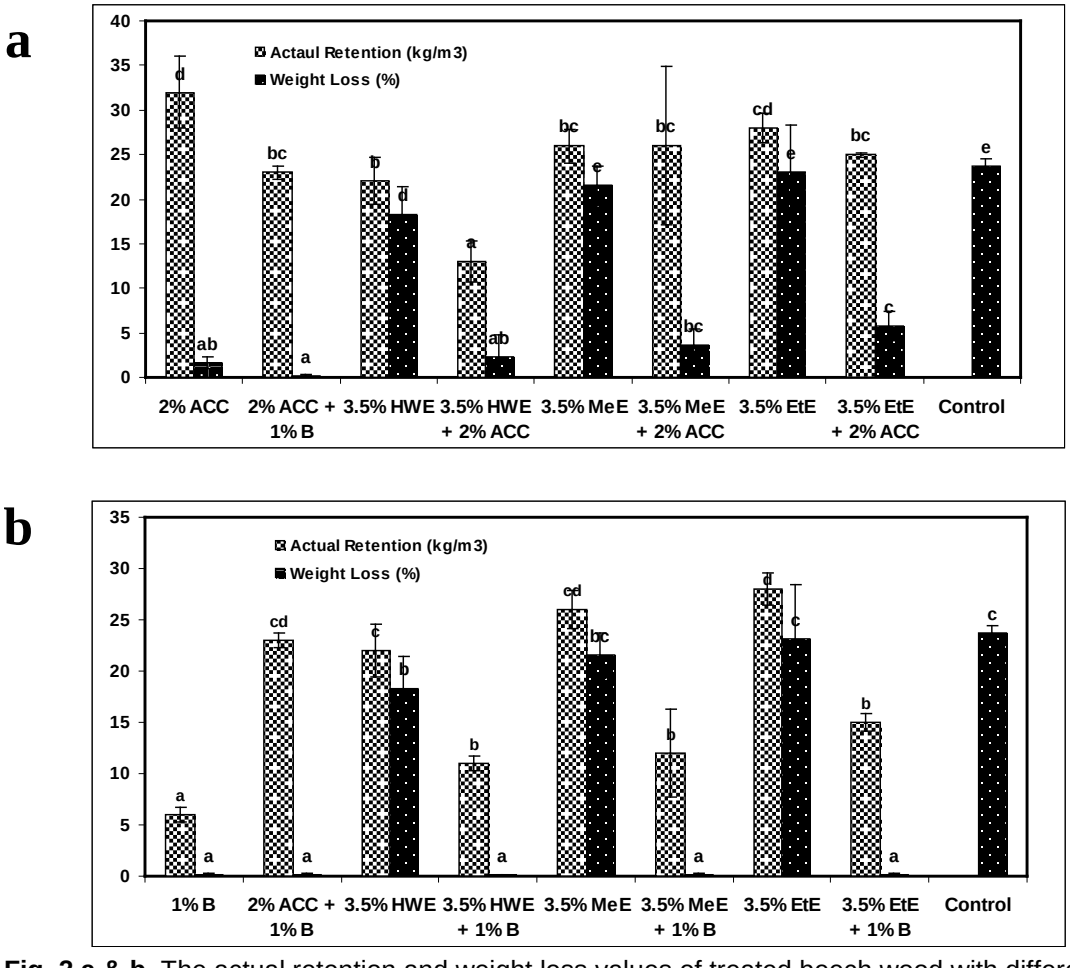
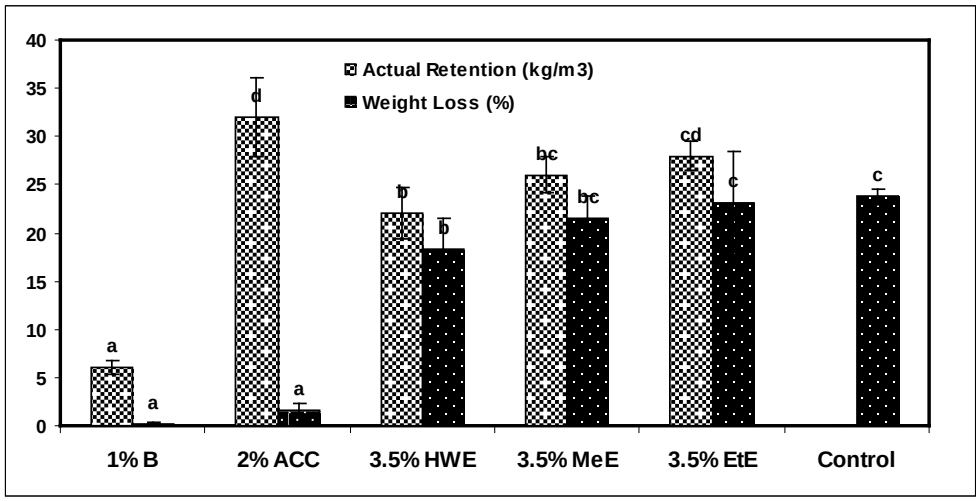


Fig. 2 a & b. The actual retention and weight loss values of treated beech wood with different chemical solutions and concentration; * Superscripts indicate statistically significant differences and Duncan ranking of the average value of measured property.



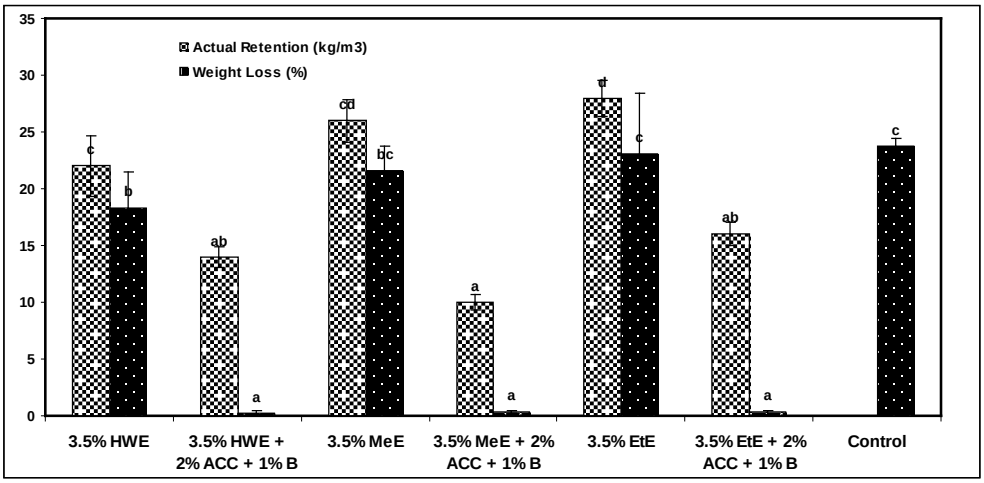


Fig. 2 c & d. The actual retention and weight loss values of treated beech wood with different chemical solutions and concentration; * Superscripts indicate statistically significant differences and Duncan ranking of the average value of measured property.

The effect of white-rot fungus mycelium growth was tested on untreated specimens, those treated with 3 different types of extract, and a combination of 3 different types of extract with ACC and B. The Willeitner scale showed distinct evidence of fungal colonization (100%) on untreated wood (Willeitner 1984). However, the individual effect of ACC, the individual effect of B, the interaction effects of ACC and B, and the interaction effects of walnut heartwood extracts and boric acid treatments of beech samples effectively prevented fungal growth (5, 0, 0, and 0%, respectively). Also, the individual effects of walnut heartwood extracts (HWE, MeE, and EtE) treatment of beech samples were not satisfactory in preventing fungal growth (67, 80, and 87%, respectively), but the interaction effects of walnut heartwood extracts (HWE, MeE, and EtE) and ACC treatment of beech samples effectively prevented fungal growth (20, 20, and 30%, respectively).

Fungal resistance was further enhanced by adding walnut heartwood extracts into the recipe (Fig. 2a, b, c, and d). However, fungal resistance was further decreased by using walnut heartwood extracts alone (Fig. 2a, b, c, and d).

Extractives provided mediocre protection against white-rot fungus. The mass losses after fungal attack were high, from 18 to 23%. As can be seen from the one-way ANOVA test (Fig 2a, b, c, and d), none of the extractives (HWE, MeE, and EtE) alone plays an important role in the wood preservation. Even if they are a little lower than that for control, an efficient wood protection is not ensured.

At best, the results showed that hot water extractives of walnut provided minimal protection against fungal attack, and the mass loss in these specimens were 18%. The methanol or ethanol extracts provided no significant (alpha = 0.05 level) protection against the white-rot fungus examined, compared to the untreated wood.

The effect of hot water-soluble extractives on fungal resistance was more obvious than that of methanol and ethanol-soluble extractives (Fig 2 a, b, c, and d). Thus it can be suggested that at 3.5% concentration of hot water-soluble extractives, fungal resistance of treated beech wood specimens is only slightly increased, and the extractives removed by hot water play a minor role in the decay resistance of the wood to white-rot fungus. It may be concluded that hot water extraction removed more of the extractives that are thought to enhance the resistance of heartwood. Some fungicidal substances, such as juglone, 2,7-dimethyl phenanthrene, and gallic acid have been shown to be removable from walnut heartwood flour (Hosseini Hashemi and Jahan Latibari 2011).

Methanol and ethanol-soluble extractives would be expected to remove materials from the cell wall, including condensed tannins, flavonoids, and phenolics (Kishino *et al.* 1995; Laks 1991); however, the present work indicated that the hot water-soluble extractives have more anti-fungal properties to a white-rot fungus *Trametes versicolor* (Huang *et al.* 2004) than the methanol and ethanol extracts.

The mass loss in the ACC- and B-treated specimens varied between 1.6 and 0.2%, respectively. Also, results in Fig. 2a and b indicate that the individual effect of ACC and the interaction effects of walnut heartwood extractives with ACC were significantly different with respect to the interaction effect of ACC and HWE. But, the effect of B alone and the combination of walnut heartwood extractives with B on the weight losses of treated samples were not significantly different together.

The fungal resistance was further increased by using 2% ACC alone, 1% B alone, and a combination of 1% B and 2% ACC. This can be attributed to the suitable anti-fungal characteristics of ACC (Su *et al.* 2007) and B (Yalincilic *et al.* 1999), and further acidification of wood samples treated with B (Yamaguchi 2001). In this study, the effect of 1% B on reduction of beech wood samples weight loss was only slightly increased by using 3.5% hot water walnut heartwood extractives. Conversely, the effect of 2% ACC on reduction of beech wood samples weight loss was only slightly decreased by using 3.5% ethanol extract of walnut heartwood. It can be postulated that water is a good bulking agent and may swell wood structure more than swelling caused by ethanol. Aromatic compounds derived from glucose such as flavonoids and condensed tannins (Ajuong and Breese 1998), usually have free hydroxyl groups and are water-soluble (Nzokou and Kamdem 2004).

When heartwood extractives were impregnated into susceptible beech wood, the level of fungal resistance was inferior (Schultz *et al.* 2008). The present results clearly demonstrated that B treatments of beech wood generally slightly outperformed treatments of wood with extracts alone. This suggests that there was not any positive combined synergistic effect of B and wood extractives.

The combination of wood extractives and B resulted in smaller mass loss than that from the B-treated wood specimens, but no significant difference between individual and interaction effects of walnut heartwood extractives on mass loss of samples.

CONCLUSIONS

1. In this study, beech sapwood specimens were used to determine the contribution of individual and interaction effects of walnut heartwood, boric acid, and acid copper chromate treatments to a white-rot fungus.
2. According to Fig. 2a, b, c, and d, it can be concluded that B and ACC treating solutions alone provided excellent wood protection. In contrast, the extractives alone did not provide acceptable protection. The combinations of extractives with wood preservatives provided efficient protection, only because of the pronounced antifungal effect of B and ACC.

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REFERENCES CITED

Ajuong, E-M. A., and Breese, C. M. (1998). "The roles of extractives on short term creep in compression parallel to grain of Pai wood (*Afzelia africana* Smith)," *Wood Fiber Sci.* 29(2), 161-170.

British Standard 838. (1961). "Methods of test for toxicity of wood preservatives to fungi," Philadelphia, PA., USA.

Cooper-Drive, G. A., and Bhattacharya, M. (1998). "Role of phenolics in plant evolution," *Phytochemistry* 49, 1165-1174.

Goodell, B., Jellison, J., Liu, J., Daniel, G., Paszcynski, A., Fekete, F., Krishnamurthy, S., Jun, L., and Xu, G. (1997). "Low molecular weight chelators and phenolic compounds isolated from wood decay fungi," *Biotechnol* 53, 133-162.

Green III, F., and Schultz, T. P. (2003). "New environmentally-benign concept in wood protection: The combination of organic biocides and non-biocidal additives," in *Wood Deterioration and Preservation*, ACS Symposium Series No. 845, Goodell, B.,

Nicholas, D., and Schultz, T. (eds.), American Chemical Society, Washington, DC, 378-389.

Hadi, S. M., Asad, S. F., Sigh, S., and Ahmad, A. (2000). "Putative mechanism for anticancer and apoptosis-inducing properties of plant-derived polyphenolic compounds," *IUMB Life* 50, 167-171.

Hosseini Hashemi, S. K., and Jahan Latibari, A. (2011). "Evaluation and identification of walnut heartwood extractives for protection of poplar wood," *BioResources* 6(1), 59-69.

Huang, Z., Maher, K., and Amartey, S. (2004). "Effects of heartwood extractives in Dahoma (*Piptadeniastrum africanum*) on decay resistance to white- and brown-rot fungi," *The International Research Group on Wood Preservation*, IRG/WP 04-10536.

Hwang, W. J., Kartal, S. N., and Imamura, Y. (2006). "Evaluation of new quaternary ammonia compound didecyldimethylammonium tetrafluoroborate (DBF) in comparison with DDAC: Leachability and termite resistance tests," *Holz als Roh und Werkstoff* 64, 111-116.

Kishino, M., Ohi, H., and Yamaguchi, A. (1995). "Characteristics of methanol extractives from Chengal wood and their antifungal properties," *Mokuzai Gakkaishi* 41, 444-447.

Laks, P. E. (1991). "The chemistry of wood bark," Pages from D. N.-S. Hon and N. Shiraishi (eds.), *Wood and Cellulosic Chemistry*, Marcel Dekker Inc., New York and Basel, 257-330.

Larson, R. A. (1988). "The antioxidants of higher plants," *Phytochemistry* 27, 969-978.

Leightley, L. E. (2003). "Protection of wood using combinations of biocides," In: *Wood Deterioration and Preservation*, ACS Symposium Series No. 845, Goodell, B., Nicholas, D., and Schultz, T. (eds.), American Chemical Society, Washington, DC, 390-398.

Lloyd, J. D. (1997). "International status of borate preservative systems," In: Proc Second International Conference on Wood Protection with Diffuse Preservatives and Pesticides, Forest Products Society, Madison, WI, 45-54.

Nzokou, P., and Kamdem, D. P. (2004). "Influence of wood extractives on moisture sorption and wettability of red oak (*Quercus rubra*), black cherry (*Prunus serotina*), and red pine (*Pinus resinosa*)," *Wood and Fiber Science* 36(4), 483-492.

Ochrymowych, J., and McOrmond, R. R. (1978). "Performance of pole-diameter posts treated with various preservatives during a quarter century of testing at Orange Park, Florida test plot," *Proc American Wood Preservers' Association* 74, 190-213.

Schultz, T. P., Harms, W. B., Fisher, T. H., McMartrey, K. D., Minn, J., and Nicholas, D. D. (1995). "Durability of angiosperm heartwood: The importance of extractives," *Holzforschung* 49, 29-34.

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

Schultz, T. P., and Nicholas, D. D. (2001). "Enhanced wood preservative composition," U.S. Patent 6,231,651.

Schultz, T. P., and Nicholas, D. D. (2002). "Development of environmentally-benign wood preservatives based on the combination of organic biocides with antioxidants and metal chelators," *Phytochemistry* 61, 555-560.

Schultz, T. P., Ragon, K., and Nicholas, D. D. (2008). "A hypothesis on a second non-biocidal property of wood extractives, in addition to toxicity, that affects termite behavior and mortality," *Document International Research Group on Wood Preservation*, IRG/Wp 08-10638.

Suttie, E. (1997). "Novel wood preservatives," *Chem. Ind.* (London) 18, 720-724.

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

Su, W-Q., Yang, D-M., and Li, J. (2007). "Experimental study on preservation performance and acute toxicity of *Maackia amurensis* Ruper.et.Maxiam. heartwood extracts," *Chemical and Industry of Forest Products* 27(04), 115-119.

Willeitner, H. (1984). "Laboratory tests on the natural durability of the timber methods and problems," *Document International Research Group on Wood Preservation*, IRG/Wp 84-2217.

Yalincilic, M. K., Gezer, E. D., Tkahashi, M., Demiric, Z., Ilhan, R., and Imamura, Y. (1999). "Boron addition to non- or low-formaldehyde cross-linking reagents to enhance biological resistance and dimensional stability of wood," *Holz als Roh- und Werkstoff* 57, 351-357.

Yamaguchi, H. (2001). "Silicic acid-boric acid complexes as wood preservatives," *International Research Group on Wood Preservation*, IRG/WP 01-30273.

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