Chemical extractives of the tropical wood Wallaba are natural anti-swelling agents

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Abstract
Wallaba (*Eperua falcata*) is a tropical wood which is known to have naturally low swelling characteristics. Samples of wallaba heartwood were subjected to differential solvent extraction. Wood pieces that were extracted with methanol experienced significantly higher swelling after rehydration from oven dry to 96% relative humidity in comparison to non-extracted samples and samples extracted with other solvents. Methanol soluble wallaba heartwood extract was purified by high pressure liquid chromatography and the compounds present were characterised by nuclear magnetic resonance spectroscopy. Methanol extract was found to contain a very high relative proportion of polar compounds which are proposed to bind to the polymeric cell wall by multiple hydrogen bonds restricting the association of water and therefore acting as natural anti-swelling agents.

Keywords: *Dimensional Stability*, Eperua falcata, *Extractives*, *HPLC*, *NMR*, *Tropical Wood*

Introduction
Wood cellulose is organized in crystalline networks of nanofibres coated by hemicellulose macromolecules and then by lignin. In the cell walls, the organization of these layers of fibre composite and the orientation of those fibres are determining factors of hygroscopic properties (Skaar 1988). Water induces dimensional variations in wood and it is present in 2 mains forms; commonly called “bound water” or “free water”. Bound water is either intimately associated into the lignocellulosic network or associated onto wood cell wall through hydrogen bonding; in the latter case this is termed “water of saturation” or “water of surface absorption.” Free water, sometimes termed “interstitial water,” is contained in cell lumens when the fibre saturation point is exceeded (approximately 30% wood moisture content). The absorbtion or desorption of free water does not influence swelling of wood (U.S. Department of Agriculture 2007). However, removal or insertion of associated water influences the organisation of wood macromolecules therefore inducing wood shrinkage or swelling (Skaar 1988).

There is a relationship between wood density and shrinkage, the study of which originates from Newlin and Wilson (1919), reviewed briefly with the other early literature in Chafe (1986). In general wood density is a direct indicator of its anatomy (Fritts 1976) and therefore its porosity. Low density woods have low wood material per unit area, so proportionality less bound water and are therefore less affected in relative dimensional changes by changing moisture content than woods with higher density and conversely proportionally more bound water. In addition to wood material however wood density can also be increased by the presence of a group of extraneous chemicals linked to heartwood formation and collectively termed extractives, which vary in quantity and composition by species (Hillis 1987). The presence of these extractives has been shown by Hernandez (2007) to be significant in the deviation of tropical hardwoods from the normally positively correlated relationship of density and shrinkage. Hernandez (2007) furthered this empirical observation deeming that the acetone and methanol extracted fractions of the studied material must contain the compounds located in the cell walls as these negatively affected, i.e. inhibited, swelling. Wood from wallaba (*Eperua falcata* Aubl., Caesalpiniaceae), a tropical rainforest species, exhibits low shrinkage (radial 2.1%; tangential 6.1%; volumetric 10.1%) during transformation from the green to the oven dry state compared to its high density of 0.86 gcm^{-3} at 12% wood moisture content (Girard and Miller 1996). Wallaba wood has also a considerable extractive content, equal to 29% the dry weight (Amusant et al. 2007), and therefore makes a good starting point for a detailed investigation into the effect of those extractives on this moisture related dimensional stability and the characterization of these compounds. This is the subject of this study.

Material and method

Sample collection and preparation

An 80 cm log was collected from each of 2 wallaba trees of approximately 40 cm diameter at breast height in Régina, French Guiana (52°752'/ 4°1857'). Boards, 2cm in the radial dimension, were cut from the logs representing outer heartwood and inner heartwood. The boards were allowed to stabilize to equilibrium moisture content in an continuously air-conditioned room before being split into bars of 20×20 mm (radial × tangential) which were then sliced lengthwise into samples of 20×20×10 mm (10 mm in longitudinal direction) size. Low variations of wood in the longitudinal direction were assumed and therefore samples separated from the same rod were considered identical, thus replications. In total for each radial position (i.e, outer heartwood and inner heartwood) 72 wood pieces were produced from each tree, these 72 wood pieces were separated in 6 groups of 12 longitudinally neighbouring samples on which to perform extraction.

Extraction of wood samples

For each tree, 5 of the 6 groups of samples were placed in an Erlenmeyer flask, each with one of five extraction solvents; 300 mL hexane, methylene chloride, ethyl acetate, methanol or water. The final group was left non-extracted to act as a control. The flasks were shaken at room temperature for 1 week. Filtration and evaporation gave the desired crude extracts of outer (hexane: 2.7%; methylene chloride: 4.2%; ethyl acetate: 6.9%; methanol: 17.5%; water: 2.1%) and inner heartwood (hexane: 2.1%; methylene chloride: 3.2%; ethyl acetate: 6.1%; methanol: 10.9%; water: 1.2%).

Volumetric swelling of selectively extracted wood pieces

Following extraction, all samples of the same group (12 pieces) were stored and air-dried at room temperature for 1 week, then dried over phosphorus pentoxide (P_2O_5) in desiccators to near 1% wood MC. At this stage, a sub-group of 3 randomly selected wood samples from each group of 12 were conditioned in chambers at 32°C over saturated salt (K_2SO_4, RH = 96%) for 10 days until they were deemed to have reached wood equilibrium moisture content

of 19%. The remaining 9 samples from each group were stabilised at other moisture contents but were not used in this manuscript (for details see Royer, 2008). Re-saturated wood samples were then weighed (W_H) on a 0.1 mg precision Sartorius balance and measured with 1 µm precision Mitutoyo comparator, in the radial (R_H) and tangential (T_H) directions. Then, wood pieces were oven-dried at 103°C for 2 days after which oven dry mass (W_0) as well as oven dry radial (R_0) and tangential (T_0) dimensions were obtained in the same way. Length in the longitudinal direction was considered as constant allowing us for calculating volumetric swelling (S) as followed:

$$S = \frac{R_H \times T_H - R_0 \times T_0}{R_0 \times T_0} \times 100 \quad (1)$$

While moisture content (MC) was calculated as followed:

$$MC = \frac{W_H - W_0}{W_0} \times 100 \quad (2)$$

Statistical analysis

The experiment contained two individual trees from which were to be drawn inferences of the population as a whole and it is unreasonable to assume that samples from the same tree were not related to each other. In short the experimental design had both fixed (extraction and wood type) and random effects with a grouping factor. Following Pinheiro and Bates (2000) a linear mixed effects model (LME) was constructed with the form:

$$G_{ijkl} = \mu + \tau_i + \upsilon_j + mc + A_k + \varepsilon_{(k)} \quad (3)$$

Where G_{ijkl} is the swelling of an individual sample, μ the overall mean, τ_i is the fixed effect of extraction i ($i = 1,2...6$), υ_j is the fixed effect of wood type j (j = 1,2), mc is the fixed effect of individual sample moisture content to account for small variations within the group, A_k is the random effect of tree k and $\varepsilon_{l(k)}$ is the random effect of sample l from tree k. An interaction was considered between extraction and wood type.

The models were examined with an F test in ANOVA (Analysis of Variance) with $\alpha = 0.05$ level of significance to determine if the effects of extraction method were significant and if the effect was different depending on the type of wood. Models were constructed in the R open source environment (R-Core Development Team 2008) using ML (Maximum Likelihood) to fit LME, a technique suited to balanced data and which allows for the comparison of models with different fixed effects. When the effect of extraction was found to be significant two alternative LME models with and without the fixed effect of extraction were compared by a likelihood ratio test in ANOVA.

Purification and Characterisation of extractives

The only extract displaying anti-swelling efficiency was the one prepared by maceration of heartwood in methanol (see figure 1 and results section). Therefore, it was chosen to conduct separation and chemical determination with this extract only. The methanol extract was evaporated to residue on a rotary evaporator. Residue was analyzed and purified by High Pressure Liquid Chromatography (HPLC). HPLC analyses were conducted using a Waters system equipped with a W600 pump and a W2996 photodiode array absorbance detector. HPLC separations were performed on a Discovery® C18 column (250 × 21.2 mm, 5 µm, Supelco®) with a linear gradient of H_2O/CH_3CN starting with a relative proportion of 80:20 and changing over 10 min to pure CH_3CN. The flow rate was 15 $mL.min^{-1}$ and the detection of compounds was operated at 300 nm. Nuclear Magnetic Resonance (NMR) structural identifications were performed on a Bruker Avance DRX500 spectrometer (^1H-500.13 MHz) equipped with a 5 mm triple resonance inverse cryoprobe TXI (^1H-^{13}C-^{15}N), with z gradient. Spectra were recorded with 1.7 mm NMR capillary tube in 40 µL of 100% CD_3OD solvent (δ_{1H} 3.31 ppm - δ_{13C} 49.00 ppm) at 300 K. The pulse programs of all 1D/2D experiments (^1H, ^{13}C-DEPTQ, COSY, NOESY, HMQC and HMBC) were taken from the Bruker standard

software library. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm cell.

Extensive purifications allowed us to isolate:

(±)-eperuic acid (**1**). **1** is transparent in UV and was detected in HPLC fractions by TLC. It has been demonstrated in the literature that biosynthesis of labdane-type diterpenes may not be stereoselective (Fukuyama et al. 1999).

Engeletin (**2**):Isoengeletin (**3**) (2.3:1): 0.40%.

Neoengeletin (**4**):Neoisoengeletin (**5**) (8.3:1): 0.22%.

Astilbin (**6**):Neoastilbin (**7**) (1.2:1): 0.15%.

p-Hydroxybenzoic acid (**8**): 0.09%.

Gallic acid (3,4,5-Trihydroxybenzoic acid, **9**): 0.16%.

(+)-Catechin (**10**):(–)-Epicatechin (**11**) (1:1.5): 1.09%. Absolute configurations were tentatively assigned based on optical rotation of epicatechin derivative **12**, assuming that **12** should be a derivative of wallaba epicatechin.

(–)-3-(4-Hydroxybenzoyl)epicatechin (**12**): 1.02%, $[\alpha]_D^{20}$ -38.4° (c 0.012, MeOH).

(–)-Dihydrokaempferol (**13**): 0.41%, $[\alpha]_D^{20}$ -56.3° (c 0.009, MeOH).

Yields reported are those obtained for the fraction used for structural elucidation. Isolation yields are not quantitative due to extensive overlap of HPLC peaks, especially for compounds **2**-**7**. In all cases, literature data confirmed identifications.

Results

Swelling

Wallaba wood pieces were extracted with different solvents and after rehydration to a mean wood moisture content of 19.04% (standard deviation = 0.78%) the volumetric swelling was measured and compared with reference non-extracted wood pieces (figure 1). The relative

significance of parameters was assessed using analysis of variance of the linear mixed effects model in equation 3 and results are listed in table 1.

In figure 1 it is clear that the samples extracted by methanol had higher swelling than samples from both the control and the other extraction methods. Analysis of variance (table 1) showed that variation in individual sample moisture content was the most significant effect (F_{158} = 93.25, $p < 0.001$) followed by wood type (F_{158} = 61.94, $p < 0.001$) then extraction (F_{558} = 6.73, $p < 0.001$). A likelihood ratio comparison of the two alternate models for swelling fitted with and without the effect of extraction showed the inclusion of extraction in the model to be significant ($p = 0.0013$). An examination of the model estimates showed that the samples that were extracted with methanol displayed significantly ($\alpha = 0.05$) higher swelling than the control and the other methods of extraction. The interaction term between extraction and wood type was not significant ($F = 0.76$, $p = 0.589$) which shows that the method of extraction did not have a different effect depending on wood type. When the difference between means was examined internal heartwood samples extracted in methanol had swollen on average 0.63% (actual not percentage difference) more than the control following whilst the external heartwood samples had swollen on average 0.82% more than the control. Mean wood density of the internal heartwood samples was 0.82 g cm^{-3} (standard deviation = 0.03g cm^{-3}) before extraction and 0.74 g cm^{-3} (standard deviation = 0.03g cm^{-3}) after methanol extraction. Mean wood density of the external heartwood samples was 0.82 g cm^{-3} (standard deviation = 0.02 g cm^{-3}) before extraction and 0.69 g cm^{-3} (standard deviation = 0.03 g cm^{-3}) after methanol extraction.

Characterisation of Extractives

Methanol is the best solvent for extraction of wallaba. In addition, its intermediate polarity makes it capable of extracting compounds usually insoluble in water or less polar solvents.

Therefore we believe that the influence of methanol extraction results both from its efficiency as well as from the very nature of methanol soluble extractives. The mass of residue obtained from the outer heartwood was equal to 17.5% of the dry mass of the samples whilst the mass of residue from the internal heartwood was equal to 10.9%. Wallaba heartwood extractives were purified and separated on HPLC whereby the chromatograms were found to be identical, in terms of composition, for internal and external heartwood (data not shown). Extractives were then identified by NMR spectroscopy, with identifications verified by published literature. Characterisation allowed us to establish that the active extract contains 13 main compounds represented in figure 2. The isolated compounds were identified as: eperuic acid (**1**, 0.31 %) (Amusant et al. 2007, Marchesini et al. 2009), engeletin (**2**, 0.28 %) (Yinrong and Yeap 1999), isoengeletin (**3**, 0.12 %) (Xu et al. 2005), a mixture of the epimers, neoengeletin and neoisoengeletin **(4** and **5**, 0.22%), a mixture of the isomers, astilbin and neoastilbin (**6** and **7**, 0.15%), *para*-hydroxybenzoic acid (**8**, 0.09 %), 3,4-dihydroxy-5-methoxy-benzoic acid **(9**, 0.16%) (Saito and Kawabata 2006), (+)-catechin (**10**, 0.44 %) (Nay et al. 2001), (–)-epicatechin (**11**, 0.65 %) (Mendoza-Wilson and Glossman-Mitnik 2006), (–)-epicatechin 3-*O*-*para*-hydroxybenzoate (**12**, 1.02 %) (Watanabe 1998) and (–)-dihydrokaempferol (**13**, 0.41 %) (Yinrong and Yeap 1999).

Discussion

The extraction of compounds soluble in methanol increased the swelling of wallaba wood pieces subjected to elevated ambient relative humidity. This result is coherent with the phenomenon observed by Hernandez (2007) who demonstrated that the extractives which induce wood swelling are soluble in polar solvents such as acetone or methanol. For the wallaba samples, those from the internal heartwood had swollen more than those from the external heartwood but the method of extraction did not have a different effect depending on

whether the wood was internal or external heartwood. This radial variability in the heartwood has been described before for two shortleaf pine trees (Choong and Fogg 1989). The difference between outer and inner heartwood may be linked to differences in extractive content (Lacandula 2002) and the subsequent effect on wood density (Hernandez, 2007). To further illustrate this point it would seem that despite having the same density the internal heartwood samples in the non-extracted group had swollen more than the external heartwood samples because they contained less extractives. Following methanol extraction the samples from the internal heartwood had swollen more than the external heartwood samples because they were denser, thus conforming to the original theory of Newlin and Wilson (1919), and confirming the proportional role of the quantity of extractives in dimensional stability.

In the light of this information, we embarked upon analysing and quantifying those wood extractives soluble in methanol in order to better apprehend the mechanisms involved in the natural lowering of wood shrinkage. Overall, methanol is able to take a wide range of compounds out of the wood. In the mixture, the major constituents are polyphenols (compounds **8**-**13**, 2.77% cumulated yield) and glycosylated polyphenols (compounds **2**-**7**, 0.77% cumulated yield). These compounds are all very polar and presumably prone to associate with the cell wall in amorphous regions of the macromolecules network, therefore contributing to the supramolecular organization of the network and competing with water absorption. This phenomenon has been proposed before by Shupe et al. (1996) in order to account for hysteresis effect observed in the dimensional changes of the wood from sweetgum (*Liquidambar styraciflua* L.) trees, although the authors did not characterize the compounds responsible for this property. Our work seems to confirm Shupe et al.'s (1996) hypothesis and demonstrates that chemicals influencing wood swelling are indeed capable of interacting with the wood cell wall.

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Figure. 1. Wallaba heartwood swelling after extraction and rehydration from oven dry to 19% wood moisture content. Control = no extraction, Hex = hexane, MC = methylene chloride, AcOEt = ethyl acetate, MeOH = methanol and H_2O = water. The methanol extracted samples displayed significantly ($\alpha = 0.05$) higher swelling than the other extraction methods. Internal heartwood samples had significantly higher swelling than external heartwood samples.

Figure 2 ***Molecules isolated in methanol extract of outer and inner heartwood of*** **Eperua falcata** ***(Rha = α-L-Rhamnopyranosyl).***

Table 1 ***Analysis of variance of the linear mixed effects model to determine the significance of extraction method and wood type (inner or outer heartwood) on the swelling of rehydrated oven dry wallaba wood samples.***

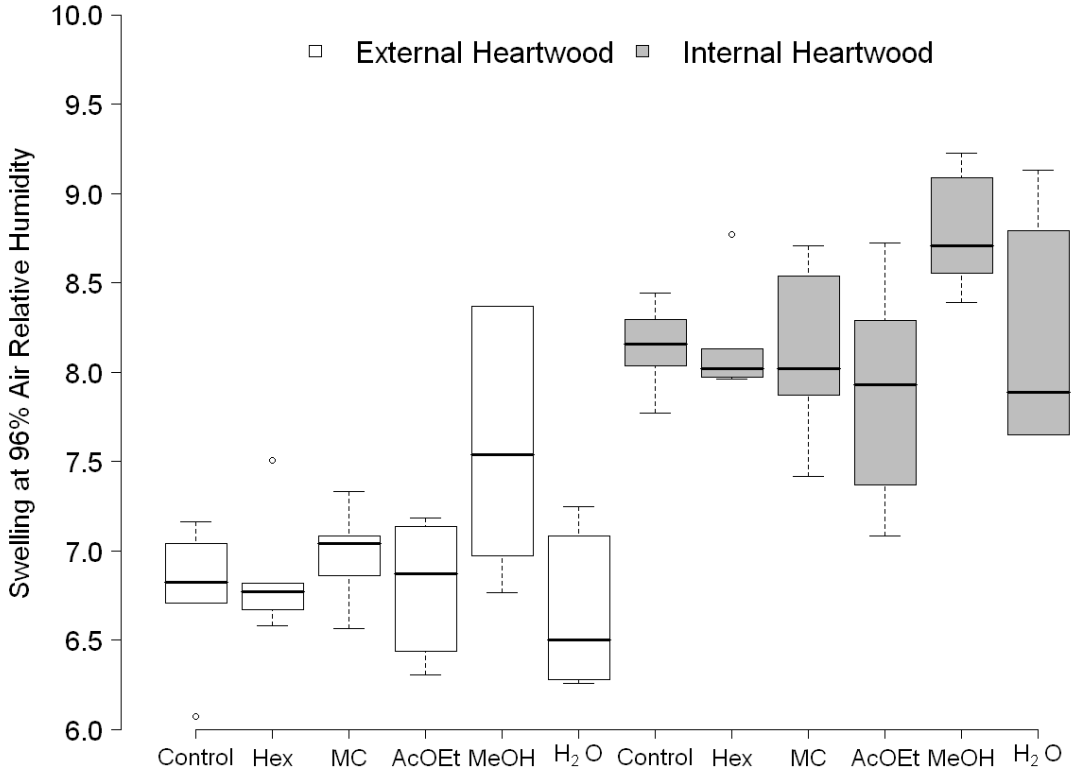
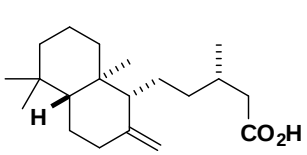
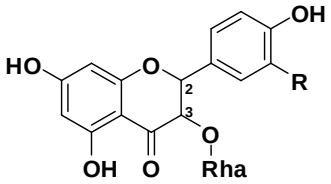


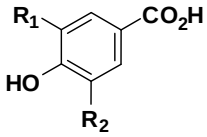
Figure 1. Wallaba heartwood swelling after extraction and rehydration from oven dry to 19% wood moisture content. Control = no extraction, Hex = hexane, MC = methylene chloride, AcOEt = ethyl acetate, MeOH = methanol and H_2O = water. The methanol extracted samples displayed significantly ($\alpha = 0.05$) higher swelling than the other extraction methods. Internal heartwood samples had significantly higher swelling than external heartwood samples.



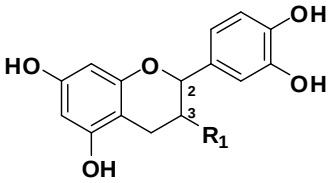
(±)-Eperuic acid (**1**): 0.31%



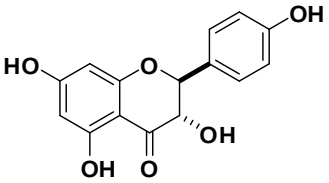
Engeletin (**2**): 2*R*,3*R*, R = H
Isoengeletin (**3**): 2*R*,3*S*, R = H } 2.3:1 (0.40%)
Neoengeletin (**4**): 2*S*,3*S*, R = H
Neoisoengeletin (**5**): 2*S*,3*R*, R = H } 8.3:1 (0.22%)
Astilbin (**6**): 2*R*,3*R*, R = OH
Neoastilbin (**7**): 2*S*,3*S*, R = OH } 1.2:1 (0.15%)



8: $R_1 = R_2 = H$ (0.09%)
9: $R_1 = OH$, $R_2 = OMe$ (0.16%)



(+)-Catechin (**10**): 2*R*,3*S*, $R_1 = OH$
(-)-Epicatechin (**11**): 2*R*,3*R*, $R_1 = OH$ } 1:1.5 (1.09%)
(-)-**12**: 2*R*,3*R*, R_1 = *p*-hydroxybenzoate (1.02%)



(-)-Dihydrokaempferol (**13**) (0.41%)

Figure 2 ***Molecules isolated in methanol extract of outer and inner heartwood of*** **Eperua falcata** ***(Rha = α-L-Rhamnopyranosyl).***

Table 1 ***Analysis of variance of the linear mixed effects model to determine the significance of extraction method and wood type (inner or outer heartwood) on the swelling of rehydrated oven dry wallaba wood samples.***

Coefficient	Numerator d.f.	Denominator d.f.	F-value	p-value
Individual Moisture Content	1	58	93.2458	<.0001
Extraction	5	58	6.7301	0.0001
Wood Type	1	58	61.9424	<.0001
Extraction x Wood Type	5	58	0.7551	0.5858