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

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15

1 **Abstract**

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

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

13

14 **Introduction**

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

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

23

24 ***Material and method***

25 Sample collection and preparation

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

12

13 Extraction of wood samples

14 For each tree, 5 of the 6 groups of samples were placed in an Erlenmeyer flask, each with one
15 of five extraction solvents; 300 mL hexane, methylene chloride, ethyl acetate, methanol or
16 water. The final group was left non-extracted to act as a control. The flasks were shaken at
17 room temperature for 1 week. Filtration and evaporation gave the desired crude extracts of
18 outer (hexane: 2.7%; methylene chloride: 4.2%; ethyl acetate: 6.9%; methanol: 17.5%; water:
19 2.1%) and inner heartwood (hexane: 2.1%; methylene chloride: 3.2%; ethyl acetate: 6.1%;
20 methanol: 10.9%; water: 1.2%).

21

22 Volumetric swelling of selectively extracted wood pieces

23 Following extraction, all samples of the same group (12 pieces) were stored and air-dried at
24 room temperature for 1 week, then dried over phosphorus pentoxide (P₂O₅) in desiccators to
25 near 1% wood MC. At this stage, a sub-group of 3 randomly selected wood samples from
26 each group of 12 were conditioned in chambers at 32°C over saturated salt (K₂SO₄, RH =
27 96%) for 10 days until they were deemed to have reached wood equilibrium moisture content

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

$$9 \quad S = \frac{R_H \times T_H - R_0 \times T_0}{R_0 \times T_0} \times 100 \quad (1)$$

10 While moisture content (MC) was calculated as followed:

$$11 \quad MC = \frac{W_H - W_0}{W_0} \times 100 \quad (2)$$

12
 13 Statistical analysis

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

$$19 \quad G_{ijkl} = \mu + \tau_i + v_j + mc + A_k + \varepsilon_{l(k)} \quad (3)$$

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

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

9

10 Purification and Characterisation of extractives

11 The only extract displaying anti-swelling efficiency was the one prepared by maceration of
12 heartwood in methanol (see figure 1 and results section). Therefore, it was chosen to conduct
13 separation and chemical determination with this extract only. The methanol extract was
14 evaporated to residue on a rotary evaporator. Residue was analyzed and purified by High
15 Pressure Liquid Chromatography (HPLC). HPLC analyses were conducted using a Waters
16 system equipped with a W600 pump and a W2996 photodiode array absorbance detector.
17 HPLC separations were performed on a Discovery[®] C18 column (250 × 21.2 mm, 5 μ m,
18 Supelco[®]) with a linear gradient of H₂O/CH₃CN starting with a relative proportion of 80:20
19 and changing over 10 min to pure CH₃CN. The flow rate was 15 mL.min⁻¹ and the detection
20 of compounds was operated at λ 300 nm. Nuclear Magnetic Resonance (NMR) structural
21 identifications were performed on a Bruker Avance DRX500 spectrometer (¹H-500.13 MHz)
22 equipped with a 5 mm triple resonance inverse cryoprobe TXI (¹H-¹³C-¹⁵N), with z gradient.
23 Spectra were recorded with 1.7 mm NMR capillary tube in 40 μ L of 100% CD₃OD solvent
24 (δ_{1H} 3.31 ppm - δ_{13C} 49.00 ppm) at 300 K. The pulse programs of all 1D/2D experiments (¹H,
25 ¹³C-DEPTQ, COSY, NOESY, HMQC and HMBC) were taken from the Bruker standard

1 software library. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter
2 equipped with a sodium lamp (589 nm) and a 1 dm cell.
3 Extensive purifications allowed us to isolate:
4 (±)-eperuic acid (**1**). **1** is transparent in UV and was detected in HPLC fractions by TLC. It
5 has been demonstrated in the literature that biosynthesis of labdane-type diterpenes may not
6 be stereoselective (Fukuyama et al. 1999).
7 Engeletin (**2**):Isoengeletin (**3**) (2.3:1): 0.40%.
8 Neoengeletin (**4**):Neoisoengeletin (**5**) (8.3:1): 0.22%.
9 Astilbin (**6**):Neoastilbin (**7**) (1.2:1): 0.15%.
10 *p*-Hydroxybenzoic acid (**8**): 0.09%.
11 Gallic acid (3,4,5-Trihydroxybenzoic acid, **9**): 0.16%.
12 (+)-Catechin (**10**):(-)-Epicatechin (**11**) (1:1.5): 1.09%. Absolute configurations were
13 tentatively assigned based on optical rotation of epicatechin derivative **12**, assuming that **12**
14 should be a derivative of wallaba epicatechin.
15 (-)-3-(4-Hydroxybenzoyl)epicatechin (**12**): 1.02%, $[\alpha]_D^{20} -38.4^\circ$ (c 0.012, MeOH).
16 (-)-Dihydrokaempferol (**13**): 0.41%, $[\alpha]_D^{20} -56.3^\circ$ (c 0.009, MeOH).
17 Yields reported are those obtained for the fraction used for structural elucidation. Isolation
18 yields are not quantitative due to extensive overlap of HPLC peaks, especially for compounds
19 **2-7**. In all cases, literature data confirmed identifications.

20

21 **Results**

22

23 Swelling

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

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

3 In figure 1 it is clear that the samples extracted by methanol had higher swelling than samples
4 from both the control and the other extraction methods. Analysis of variance (table 1) showed
5 that variation in individual sample moisture content was the most significant effect ($F_{158} =$
6 93.25 , $p < 0.001$) followed by wood type ($F_{158} = 61.94$, $p < 0.001$) then extraction ($F_{558} =$
7 6.73 , $p < 0.001$). A likelihood ratio comparison of the two alternate models for swelling fitted
8 with and without the effect of extraction showed the inclusion of extraction in the model to be
9 significant ($p = 0.0013$). An examination of the model estimates showed that the samples that
10 were extracted with methanol displayed significantly ($\alpha = 0.05$) higher swelling than the
11 control and the other methods of extraction. The interaction term between extraction and
12 wood type was not significant ($F = 0.76$, $p = 0.589$) which shows that the method of
13 extraction did not have a different effect depending on wood type. When the difference
14 between means was examined internal heartwood samples extracted in methanol had swollen
15 on average 0.63% (actual not percentage difference) more than the control following whilst
16 the external heartwood samples had swollen on average 0.82% more than the control. Mean
17 wood density of the internal heartwood samples was 0.82 g cm^{-3} (standard deviation = 0.03 g
18 cm^{-3}) before extraction and 0.74 g cm^{-3} (standard deviation = 0.03 g cm^{-3}) after methanol
19 extraction. Mean wood density of the external heartwood samples was 0.82 g cm^{-3} (standard
20 deviation = 0.02 g cm^{-3}) before extraction and 0.69 g cm^{-3} (standard deviation = 0.03 g cm^{-3})
21 after methanol extraction.

22

23 *Characterisation of Extractives*

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

1 Therefore we believe that the influence of methanol extraction results both from its efficiency
2 as well as from the very nature of methanol soluble extractives. The mass of residue obtained
3 from the outer heartwood was equal to 17.5% of the dry mass of the samples whilst the mass
4 of residue from the internal heartwood was equal to 10.9%. Wallaba heartwood extractives
5 were purified and separated on HPLC whereby the chromatograms were found to be identical,
6 in terms of composition, for internal and external heartwood (data not shown). Extractives
7 were then identified by NMR spectroscopy, with identifications verified by published
8 literature. Characterisation allowed us to establish that the active extract contains 13 main
9 compounds represented in figure 2. The isolated compounds were identified as: eperuic acid
10 (**1**, 0.31 %) (Amusant et al. 2007, Marchesini et al. 2009), engeletin (**2**, 0.28 %) (Yinrong and
11 Yeap 1999), isoengeletin (**3**, 0.12 %) (Xu et al. 2005), a mixture of the epimers, neoengeletin
12 and neoisoengeletin (**4** and **5**, 0.22%), a mixture of the isomers, astilbin and neoastilbin (**6** and
13 **7**, 0.15%), *para*-hydroxybenzoic acid (**8**, 0.09 %), 3,4-dihydroxy-5-methoxy-benzoic acid (**9**,
14 0.16%) (Saito and Kawabata 2006), (+)-catechin (**10**, 0.44 %) (Nay et al. 2001), (-)-
15 epicatechin (**11**, 0.65 %) (Mendoza-Wilson and Glossman-Mitnik 2006), (-)-epicatechin 3-*O*-
16 *para*-hydroxybenzoate (**12**, 1.02 %) (Watanabe 1998) and (-)-dihydrokaempferol (**13**, 0.41
17 %).
18

19 *Discussion*

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

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

11 In the light of this information, we embarked upon analysing and quantifying those wood
12 extractives soluble in methanol in order to better apprehend the mechanisms involved in the
13 natural lowering of wood shrinkage. Overall, methanol is able to take a wide range of
14 compounds out of the wood. In the mixture, the major constituents are polyphenols
15 (compounds **8-13**, 2.77% cumulated yield) and glycosylated polyphenols (compounds **2-7**,
16 0.77% cumulated yield). These compounds are all very polar and presumably prone to
17 associate with the cell wall in amorphous regions of the macromolecules network, therefore
18 contributing to the supramolecular organization of the network and competing with water
19 absorption. This phenomenon has been proposed before by Shupe et al. (1996) in order to
20 account for hysteresis effect observed in the dimensional changes of the wood from sweetgum
21 (*Liquidambar styraciflua* L.) trees, although the authors did not characterize the compounds
22 responsible for this property. Our work seems to confirm Shupe et al.'s (1996) hypothesis and
23 demonstrates that chemicals influencing wood swelling are indeed capable of interacting with
24 the wood cell wall.

25

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1 **Figure. 1.** Wallaba heartwood swelling after extraction and rehydration from oven dry to 19%
2 wood moisture content. Control = no extraction, Hex = hexane, MC = methylene chloride,
3 AcOEt = ethyl acetate, MeOH = methanol and H₂O = water. The methanol extracted samples
4 displayed significantly ($\alpha = 0.05$) higher swelling than the other extraction methods. Internal
5 heartwood samples had significantly higher swelling than external heartwood samples.

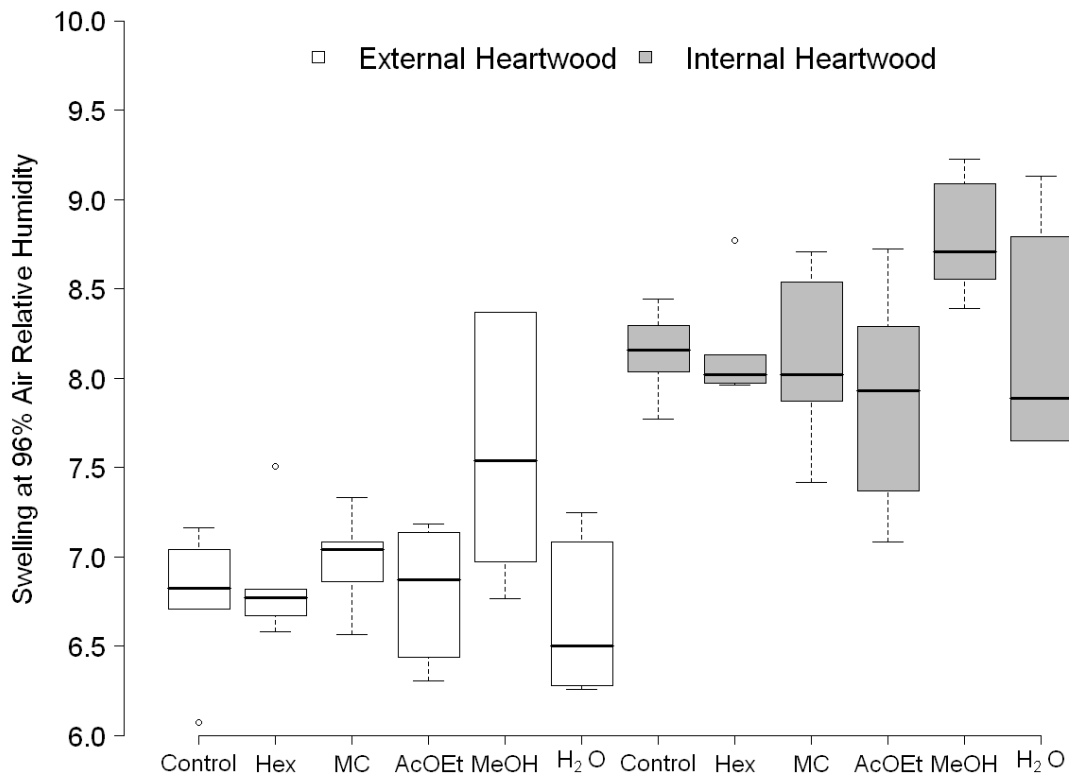
6

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

9

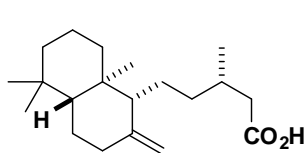
10 *Table 1 Analysis of variance of the linear mixed effects model to determine the significance*
11 *of extraction method and wood type (inner or outer heartwood) on the swelling of*
12 *rehydrated oven dry wallaba wood samples.*

13

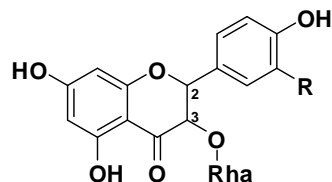


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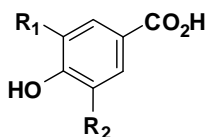
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(±)-Eperuic acid (1): 0.31%

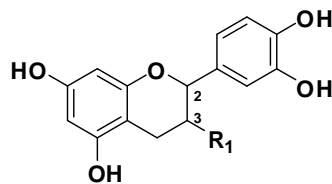


Engeletin (2): 2*R*,3*R*, R = H }
 Isoengeletin (3): 2*R*,3*S*, R = H } 2.3:1 (0.40%)
 Neoengeletin (4): 2*S*,3*S*, R = H }
 Neoisoengeletin (5): 2*S*,3*R*, R = H } 8.3:1 (0.22%)
 Astilbin (6): 2*R*,3*R*, R = OH }
 Neoastilbin (7): 2*S*,3*S*, R = OH } 1.2:1 (0.15%)



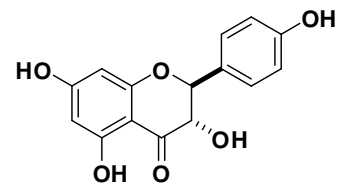
8: R₁ = R₂ = H (0.09%)

9: R₁ = OH, R₂ = OMe (0.16%)



(+)-Catechin (10): 2*R*,3*S*, R₁ = OH }
 (-)-Epicatechin (11): 2*R*,3*R*, R₁ = OH } 1:1.5 (1.09%)

(-)-12: 2*R*,3*R*, R₁ = *p*-hydroxybenzoate (1.02%)



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

1
2

3 **Figure 2 Molecules isolated in methanol extract of outer and inner heartwood of *Eperua***

4 ***falcata* (Rha = α-L-Rhamnopyranosyl).**

5

1 *Table 1 Analysis of variance of the linear mixed effects model to determine the significance*
 2 *of extraction method and wood type (inner or outer heartwood) on the swelling of*
 3 *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

4