LOCATION OF EXTRACTIVES AND DECAY RESISTANCE IN SOME MALAYSIAN HARDWOOD SPECIES

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YAMAMOTO, K. & HONG, L. T. 1989. Location of extractives and decay resistance in some Malaysian hardwood species. The durability of 24 Malaysian hardwoods has been assessed by a modified ASTM D2017 soil-block method using the white rot fungus, *Coriolus versicolor*. Using cluster analysis, the 24 timbers have been classified into four groups, *viz* durable, durable but not after extraction, moderately durable, and non-durable. In general, timbers of the durable group (*e.g.* chengal, giam, rengas) contain more extractives than the non-durable group (perupok, jelutong, ramin, rubberwood). The extractives are predominantly present in the parenchyma cells with some in the adjacent fibre cells. A higher proportion of extractives exist in parenchyma cells in the durable group. The extractives located in the cell lumina are easier to extract from the durable group than the moderately durable group.

Key words: *Coriolus versicolor* - tropical hardwood - extractives - decay - parenchyma cell - location

Introduction

Extractives are known to be associated with the decay resistance of wood (Scheffer & Cowling 1966). The amount of extractives sometimes indicates the degree of resistance of wood to decay (Takahashi & Kishima 1973, Yatagai & Takahashi 1980). A number of heartwood extractives have been tested for their anti-fungal activity (Rudman 1962, Kondo & Imamura 1986, Hong 1986, Syafii *et al.* 1987, Yamamoto & Hong 1988). The amount of extractives varies greatly, in particular, among tropical species (Buckley 1932, Peh *et al.* 1986). However, effects of extractives on decay resistance in relation to their location are not clear among tropical hardwood species. The actual location of extractives in wood cells has not been well clarified because it is sometimes difficult to prevent the elution of extractives during preparations for microscopic studies.

Therefore few studies to detect the location of extractives in relation to wood structure have been reported (Hillis 1962).

In this report the location of wood extractives was examined by light microscopy before and after their removal from wood specimens. The location of extractives and their relationship to durability as measured by decay resistance based on weight losses of both extracted and unextracted specimens were also examined.

Materials and methods

Specimens

Twenty four hardwood species from Peninsular Malaysia were examined. Only heartwood was used except for jelutong, white meranti, perupok, ramin and rubberwood in which the sapwood and heartwood are not well differentiated (Balan Menon 1967).

Extraction

Hot water extraction of four wood block samples ($20 \times 20 \times 5$ *mm*) in 150 *ml* distilled water was carried out for 6 *h* at 104°*C* in an autoclave. Methanol extraction was similarly carried out in a soxhlet extractor using paper thimbles with 150 *ml* of the solvent. After extraction each wood block sample was weighed to obtain the weight difference for determining the extractive content. Extractive contents from milled wood flour of each wood species were also determined by the same method for comparison.

Fungal decay test

A modification of the ASTM D2017 (ASTM 1986) was used for the decay test. Three replicates each of the control samples, hot water extracted samples and methanol extracted samples were subjected to decay by a white-rot fungus, *Coriolus versicolor* (L. ex Fr.) Quel, at 25°*C* for 12 weeks. Three blocks were placed into each culture glass jar for decay. The procedure used has been described in a previous paper (Yamamoto & Hong 1988). At the end of the test period, any mycelium present was removed from the surfaces of the test specimens and the specimens were oven-dried at 60°*C* for 48 *h* and reweighed to determine the weight loss.

Microscopic observation

For detecting the location of extractives, unembedded (to prevent elution of

extractives) control and extracted wood blocks were sectioned. The thickness of sections was limited to about 15 μm by using a sliding microtome. Sections without staining were directly mounted using a mixture of Canada balsam and xylene (9:1) as the mounting medium and observed with a light and a phase-contrast microscope. Decayed wood blocks were embedded with epoxy resin (Quetol 812) and sectioned to 2 μm thick by glass knives using a rotary microtome. The semi-thin sections were stained with 0.05% basic fuchsin aqueous solution for 2 *min* (Humphrey & Pittman 1974). Characteristics of decayed wood structures were determined using a light microscope.

Results and discussion

The extractives of the 24 timbers were more readily extracted from wood flour than from wood blocks (Table 1). There was no consistent difference in extractives between block and flour but timbers with low extractive contents had lower differences than those with high content of extractives (Table 1). The yield of methanol extractives from wood blocks approached that of hot water extractives from wood flour in some durable timbers like chengal, merbau and balau (Table 1).

Table 1. Hot water and methanol extractives contents from wood blocks and wood flour of 24 Malaysian hardwoods

Number	Trade Name	Scientific Name	Hot water extractives content (%)		Methanol extractives content (%)	
			Block	Flour	Block	Flour
1	Balau	*Shorea* sp.	1.9	7.4	5.2	11.4
2	Chengal	*Neobalanocarpus heimii*	10.0	23.0	29.8	32.6
3	Giam	*Hopea* sp.	6.9	16.8	10.5	22.3
4	Keranji	*Dialium* sp.	1.6	5.6	1.8	6.3
5	Merbau	*Intsia palembanica*	12.5	16.5	17.1	19.1
6	Resak	*Vatica* sp.	2.3	9.0	15.0	16.5
7	Kapur	*Dryobalanops aromatica*	3.5	5.5	4.0	6.7
8	Kempas	*Koompasia malaccensis*	0.7	2.2	1.6	3.7
9	Keruing	*Dipterocarpus* sp.	1.4	2.2	3.3	4.0
10	Mata ulat	*Kokoona* sp.	0.7	1.9	3.5	4.2
11	Punah	*Tetramerista glabra*	3.7	6.1	3.6	7.8
12	Rengas	ANACARDIACEAE	3.7	13.7	14.7	20.6
13	Bintangor	*Calophyllum* sp.	1.5	2.3	2.8	3.8
14	Durian	*Durio* sp.	0.8	2.1	5.0	4.8
15	Jelutong	*Dyera costulata*	2.5	3.8	2.7	5.8
16	Meranti bakau	*Shorea rugosa*	2.1	3.3	2.6	4.6
17	Meranti, dark red	*Shorea* sp.	1.0	2.1	2.1	4.9
18	Meranti, white	*Shorea* sp.	1.2	2.8	2.6	3.8
19	Meranti, yellow	*Shorea* sp.	3.1	3.8	4.5	8.3
20	Merawan	*Hopea* sp.	3.4	7.2	8.1	9.3
21	Mersawa	*Anisoptera* sp.	1.6	2.3	2.9	5.5
22	Perupok	*Lophopetalum* sp.	1.6	2.3	1.6	3.2
23	Ramin	*Gonystylus* sp.	2.0	2.8	1.8	3.1
24	Rubberwood	*Hevea brasiliensis*	3.1	4.6	2.0	5.5

Note: Numbers 1-6 = heavy hardwood, 7-12 = medium hardwood, 13-24 = light hardwood

The loss in weights of the unextracted and extracted wood blocks caused by *C. versicolor* are shown in Table 2. In general, extracted wood blocks suffered higher weight losses than the unextracted ones, except for 13 timbers after hot-water extraction and nine timbers after methanol extraction which had only slight increase in weight loss (<5%) compared with the unextracted blocks (Table 2). The greatest differences in weight loss between, before and after extractions for both solvents of hot water and methanol were found for merbau and merawan. Table 2 shows a general trend of the heavy and also some medium density hardwoods (Balan Menon 1967) which generally had less weight loss differences than the light hardwoods. Rengas was not decayed by *C. versicolor* at all. Hence an attempt was made to apply a flexible method of cluster analysis using squared Euclidean distance as definition of dissimilarity to classify the effect of extractives against fungal decay resistance by using data in Tables 1 and 2.

Table 2. Weight loss of unextracted and extracted wood blocks caused by *Coriolus versicolor*

Trade Name	Weight loss (%)		
	Unextracted	Hot water extracted	Methanol extracted
Balau	1.2	2.3*	3.9*
Chengal	1.4	1.7*	9.3
Giam	1.7	1.5*	5.4*
Keranji	0.3	0.5*	0.7*
Merbau	3.8	22.7	42.5
Resak	0.3	1.8*	18.6
Kapur	5.3	17.1	21.1
Kempas	8.7	14.9	11.7*
Keruing	15.3	13.6*	23.3
Mata ulat	17.7	23.2	28.5
Punah	21.4	27.5	26.2*
Rengas	0.0	0.0*	0.0*
Bintangor	18.5	25.7	40.3
Durian	19.5	19.8*	31.4
Jelutong	31.6	35.9*	38.0
Meranti bakau	11.1	27.9	25.8
Meranti, dark red	14.5	20.6	13.3*
Meranti, white	36.1	38.2*	42.6
Meranti, yellow	30.5	40.4	37.8
Merawan	1.8	24.5	58.9
Mersawa	14.6	25.7	23.8
Perupok	47.7	49.3*	50.7*
Ramin	35.8	38.0*	37.9*
Rubberwood	36.9	36.0*	43.2

* Samples with less than 5% difference from unextracted control

Figure 1 is a dendrogram of 24 species analysed, based on three factors, methanol extractive contents, weight loss of unextracted blocks and weight loss of methanol extracted blocks. The 24 species used in this experiment have been classified into four groups. The first cluster, the durable group which retained

their durability even after extraction consisted of chengal, keranji, rengas, balau, giam and resak. The second cluster which was durable but had their durability reduced after extraction consisted of merbau and merawan. The third cluster, the moderately durable group where the effect of extractives on decay resistance was not so clear consisted of kapur, kempas, keruing, mata ulat, punah, bintangor, mersawa, meranti bakau,dark red meranti and durian. The fourth cluster, the non-durable group, where the extractives had little contribution to decay resistance, consisted of jelutong, yellow meranti, ramin, rubberwood, white meranti and perupok.

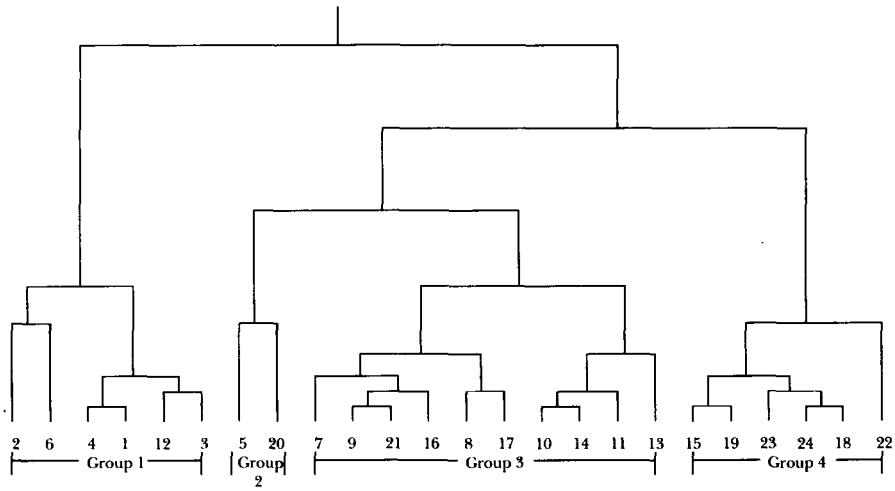


Figure 1. Cluster analysis of 24 hardwood species based on methanol extractive content and weight loss by *Coriolus versicolor* (The numbers follow species numbers in Table 1)

Microscopic observations showed that the extraneous materials located in cell lumina of many species disappeared after methanol extraction indicating that these materials were probably extractives. Chengal, one species of the first cluster was found to have extractives mainly in the lumina of axial and ray parenchyma cells (Figure 2) and the extractives were completely removed after methanol extraction (Figure 3). Another species of this cluster has also been observed to have extractives in similar locations. These observations indicated that a large proportion of extractives of the parenchyma cells is located in the lumina and the remainder in the cell walls in the durable group. Most of the parenchyma cells in the methanol-extracted chengal (9.3% weight loss) were decayed (Figure 5) when compared to the unextracted blocks (Figure 4) lending evidence to the location of extractives in the axial and ray parenchyma. The slight decrease in decay resistance after extraction of timbers in this group could be explained by the presence of guaiacyl-rich lignin in cell walls because syringyl elements of lignin are known to be more rapidly degraded than guaiacyl-rich lignin (Syafii *et al.* 1988). Highley (1982) has shown that differences in the type of lignin apparently are a key factor in the

slower degradation of woods with guaiacyl lignin by *C. versicolor*. Methanol extractives themselves have been shown to contribute to the decay resistance of chengal (Yamamoto & Hong 1988).

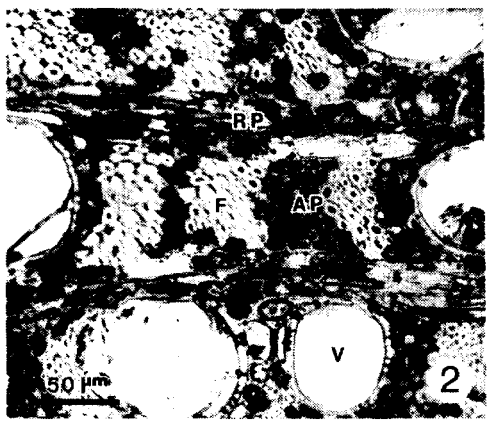
Merbau, a representative of the second cluster (durable but reduced after extraction) was observed to possess extractives generally in the lumina of both parenchyma cell types and in some fibres near the ray cells (Figures 6 & 7). The parenchyma cells and some fibres of methanol-extracted merbau blocks with 42.5% weight loss after decay attack were severely degraded when compared to unextracted blocks (Figures 8 & 9). The rapid decrease of durability after extraction could be due to the thorough extraction or a predominance of syringyl-rich over guaiacyl-rich lignin (Highley 1982, Syafii *et al.* 1988). The two timbers in this groups, merbau and merawan had the greatest difference in weight loss when extractives were removed by extraction (Table 2).

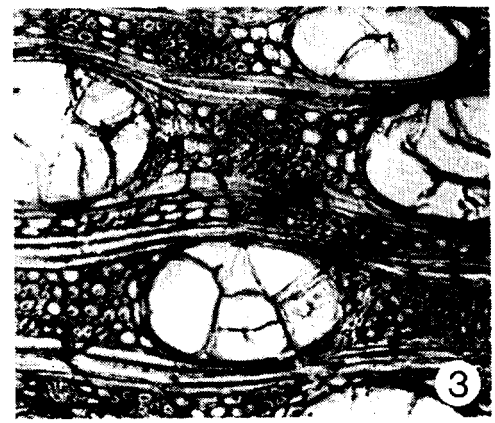
The extractives in meranti bakau, a representative of the third cluster (moderately durable) was observed predominantly in the lumina of ray parenchyma cells with some in axial parenchyma cells (Figure 10). These extractives remained even after extraction. Methanol-extracted blocks of meranti bakau with 25.8% weight loss had greater degradation in the axial parenchyma cells surrounding vessels than the other elements (Figure 11) when compared to unextracted blocks. These results suggest that extractives located in parenchyma cell lumina do not contribute much to durability in this group.

Jelutong, a species from the fourth cluster (non-durable) did not have extraneous materials in the lumina of any cell types. Unextracted and methanol extracted blocks of jelutong had similar decay features where the ray and axial parenchyma cells were more selectively decayed than the fibers (Figures 12 & 13). The methanol extractives in the timber of this group seemed to have no influence on the decay resistance.

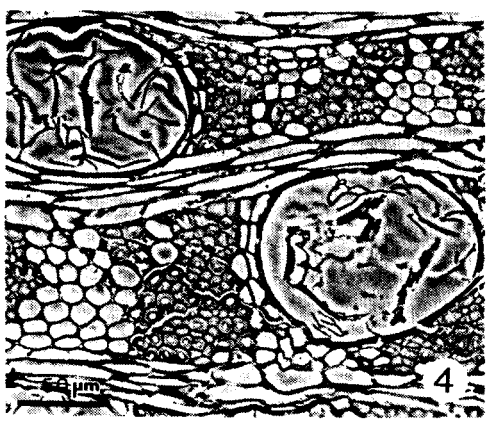
Therefore in all the timbers examined extractives were predominantly found in the axial and ray parenchyma cells with small amounts in adjacent fibres. Some of these extractives contributed to the decay resistance while others did not depending on the timber species.

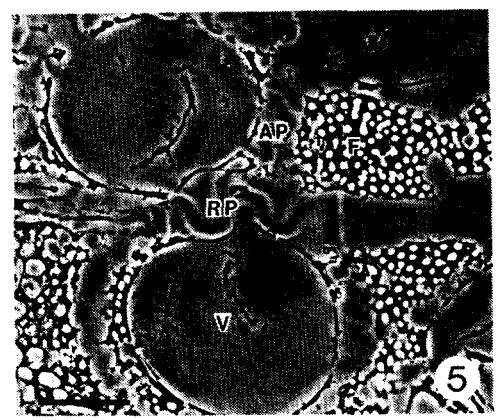
Extractives in general are found not only in cell lumina but also in cell walls. In this study, however, it was not possible to determine extractives located in cell walls. Imagawa & Fukazawa (1978) using UV absorbance at 280 *nm* had shown that cell walls of unextracted *Larix leptolepis* had 1.3 - 1.6 times more absorbance than samples successively extracted with *n*-hexane, alcohol-benzene, acetone and water. However, Bauch *et al.* (1974) reported that UV microspectrophotometrical measurements on sections pre-extracted with benzene-ethanol differed only slightly from those sections in the green condition indicating the short comings of this method for locating extractives in cell walls. Therefore, there is a need to examine and use appropriate techniques to detect the presence of extractives to ascertain their locations especially with reference to extractives with decay resistance properties.



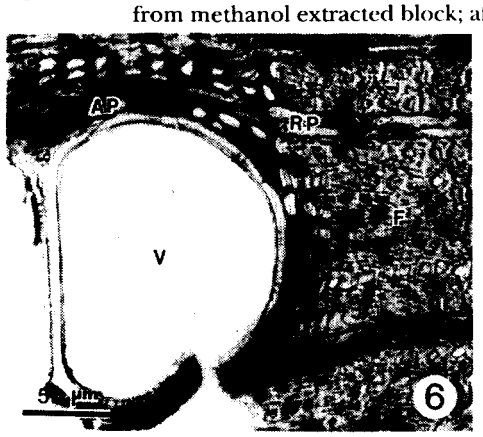


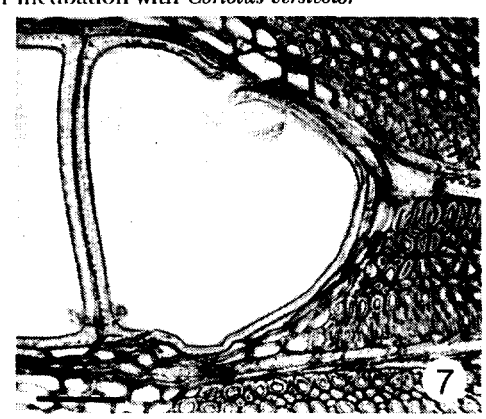
Figures 2 & 3. Cross section of chengal: (**2**) presence of extractives mainly in the lumina of axial ray parenchyma from unextracted block; (**3**) disappearance of extractives after methanol extraction [axial parenchyma cell (AP), fibre (F), ray parenchyma cell (RP), vessel (V); notations follow for other figures]



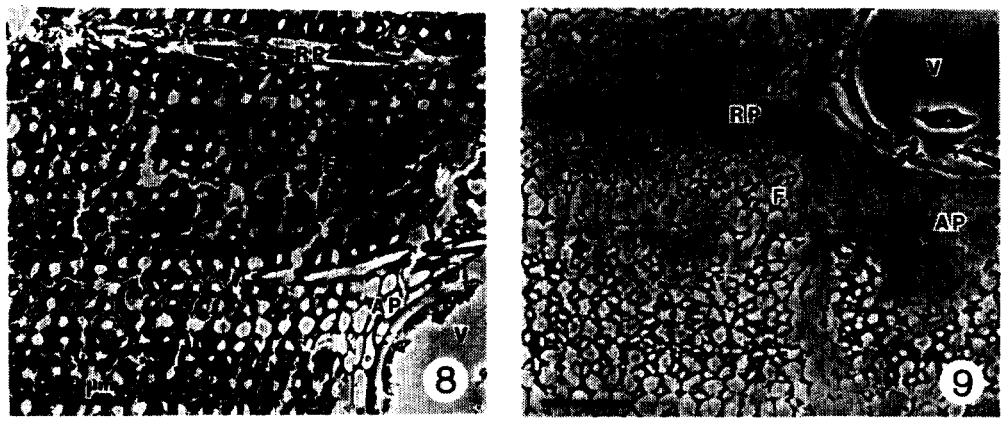


Figures 4 & 5. Phase-contrast photomicrographs of chengal cross section: (**4**) relatively undecayed sample of unextracted chengal; (**5**) decayed sample with mainly the parenchyma cells removed from methanol extracted block; after incubation with *Coriolus versicolor*

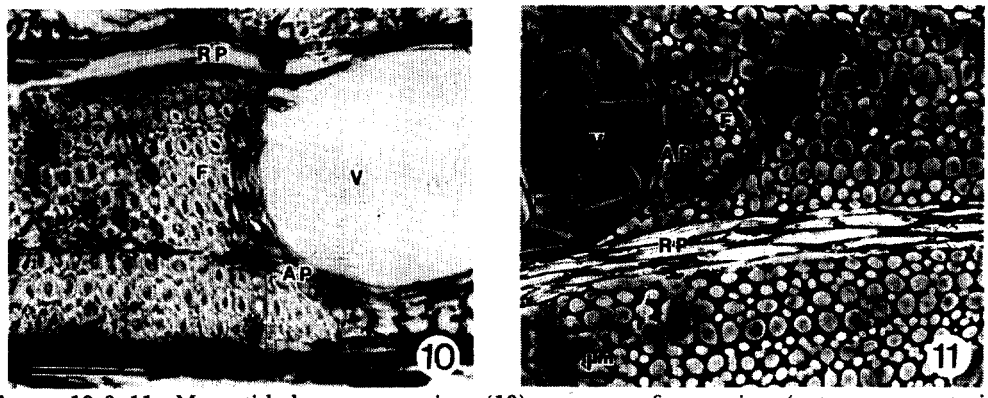




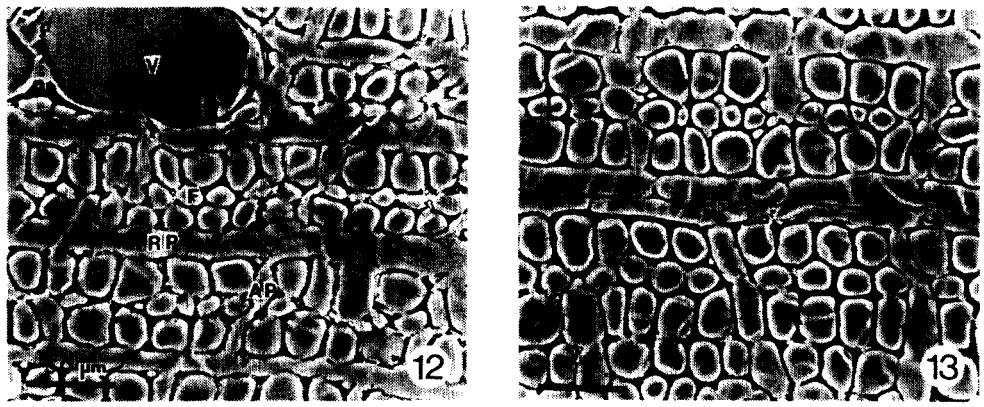
Figures 6 & 7. Cross section of merbau: (**6**) presence of extractives mainly in the parenchyma and some adjacent fibres of unextracted block; (**7**) absence of extractives in methanol extracted samples



Figures 8 & 9. Phase-contrast photomicrographs of merbau cross section: (**8**) slight degradation of cell elements in unextracted samples; (**9**) severe degradation of axial and ray parenchyma cells in methanol extracted block with 42.5% weight loss; after incubation with *Coriolus versicolor*



Figures 10 & 11. Meranti bakau cross section: (**10**) presence of extractives (extraneous material) mainly in the ray parenchyma cells of unextracted block; (**11**) phase-contrast cross section of methanol extracted sample at 25.8% weight loss; after incubation with *Coriolus versicolor* (note degradation in ray and axial parenchyma)



Figures 12 & 13. Phase-contrast photomicrographs of jelutong cross section: (**12**) degradation of cell elements at 31.6% weight loss; (**13**) degradation of cell elements at 38% weight loss of methanol extracted sample (both parenchyma cells severely degraded)

Conclusion

The cluster analysis classified the 24 species of timbers examined into four groups, *viz* durable, durable but not durable after extraction, moderately durable and non-durable based on methanol extractive contents, weight losses of unextracted and methanol extracted specimens.

Extractives were observed predominantly in cell lumina of axial and ray parenchyma. The durable species tend to possess a higher proportion of extractives.

In the durable group, extractives located in cell lumina were easy to extract with both hot water and methanol. On the other hand, in the moderately durable group, extraneous materials located in cell lumina were difficult to remove by both extractions.

The location of extractives and their behaviour towards polar solvents (*e.g.* water, methanol) could determine the degree of 'durability' of traditionally durable timbers. Hence durable timbers with higher amounts of extractives may not necessarily have higher durability when put into service.

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