COST Action FP1303
Technical Workshop
"Design, Application and Aesthetics of biobased building materials"

Sofia, Bulgaria
28 February – 1 March, 2017

Potential use of plant extracts for protection of wood veneers

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BACKGROUND

✓Kingdom ***Plantae*** is a huge source of active substances that could be used to replace some hazardous synthetic products or to increase the degradability range of some products.

✓In plants, the secondary metabolites are present in important functions such as protection and species interactions, but are not necessary for survival.

✓**Extractives** are a large number of diverse substances mainly with low molecular masses, some are valuable raw materials for making organic chemicals.

✓**Phenolic compounds** are one of the most extensive extractive groups.

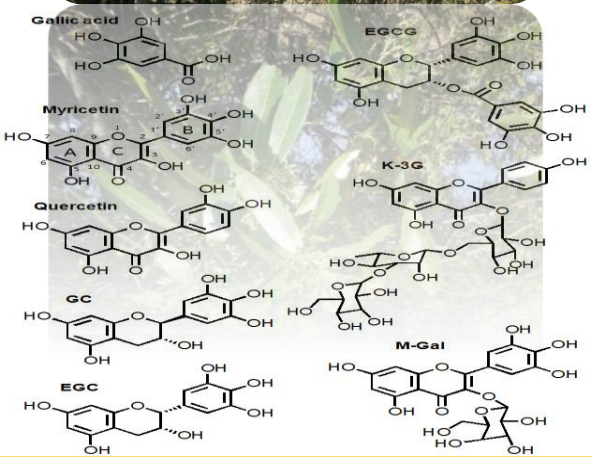
BACKGROUND

- Within the approach of **multiple-use of natural forests,** besides the need to find alternatives to **residues from the forest industry**, researchers are looking for new applications, such as their use as phytochemicals, bio-preservatives, additives, bonding materials, etc.

- ⚠ However, there are certain **limitations**, including specific legislation remarks, fixation and leachability rates as well as toxicological aspects of the extractives that could delay the commercialization of this kind of plant-based products.

BACKGROUND

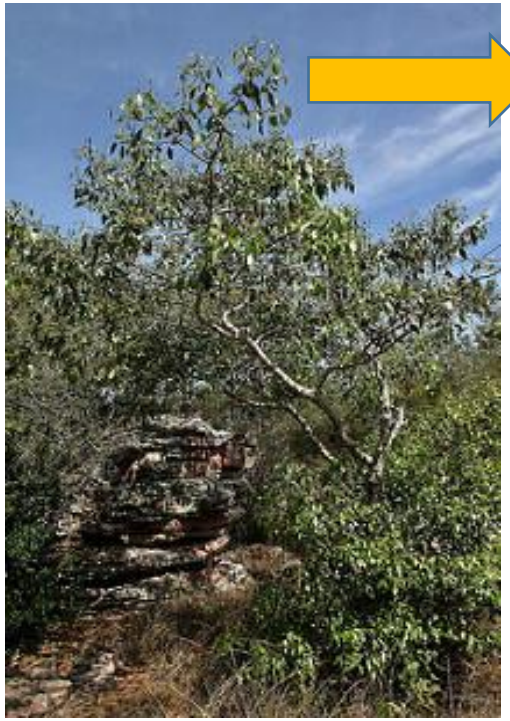
- A constant concern in industry is to find alternative methods to prolong service life of wood and its products against biological degradation in addition to preserve the environment.



- Several studies have been conducted to evaluate the efficacy against wood rot fungi of the phenolic extractives.

AIM OF STUDY

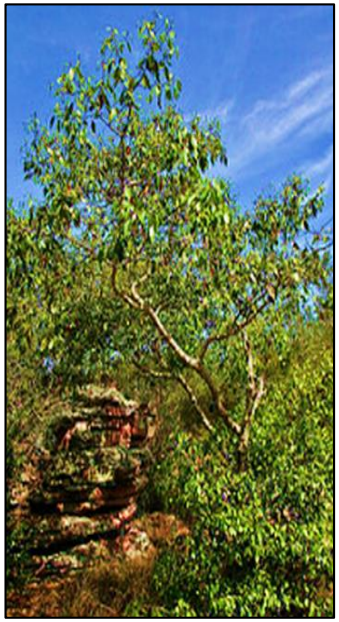
- the present study aims are to obtain the leaf crude extract, with its phenolic-rich fractions, from *Ocotea lancifolia* (Schott) Mez, and to perform the extract's detailed characterization in order to find its application as wood veneers preserving bio-agent.



It is important to obtain these value-added products through sustainable forest management.

OVERVIEW

EXTRACTION → **FRACTIONATION** → **APPLICATION** → **EVALUATION**

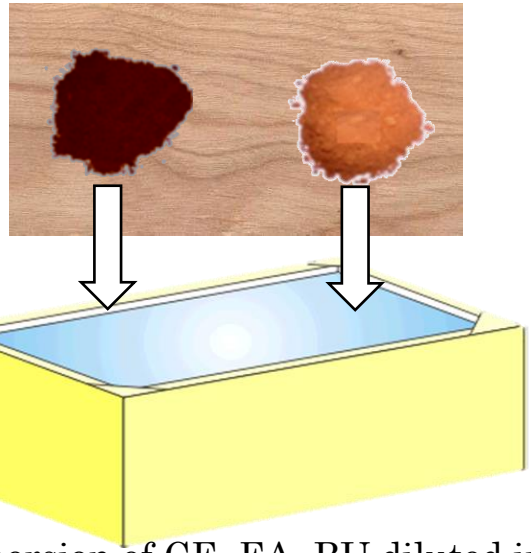


Ocotea lancifolia tree

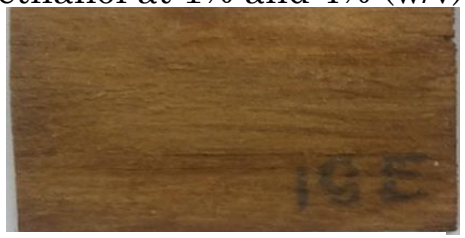
Soxhlet extraction (1:20 w/w)

L/L fractionation

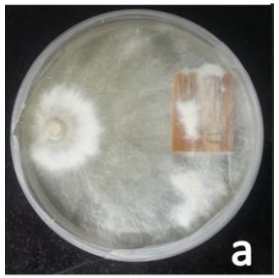
Ethyl acetate and buthanolic fractions

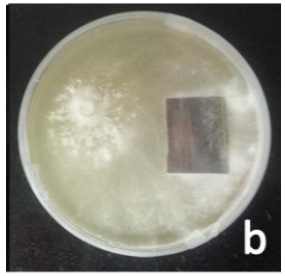


Immersion of CE, EA, BU diluted in ethanol at 1% and 4% (w/v)



Weight percentage gain (WPG)





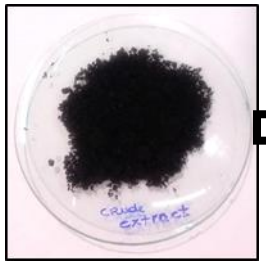
Test against *T. versicolor* and *G. trabeum*

EXTRACTION

- Leaves of *Ocotea lancifolia* (Shott) Mez were collected in Rio Grande do Sul, Brazil

Leaves were air-dried and passed through 40-mesh screen

- Soxhlet extraction with ethanol (1:20 w/w) under reflux.



The crude extract was fractionated (*L/L*) with solvents of increasing polarity

Ethyl acetate (EA) and buthanolic (BU) fractions

CHARACTERIZATION

- Total phenolic compounds, flavonoids and condensed tannins contents of *Ocotea lancifolia* leaf extract and its fractions.

Sample	Tannin content [mg CAE/g]	Flavonoid content [mg QE/g]	Total phenolic content [mg GAE/g]
Crude extract	157.60 ± 2.60	83.94 ± 1.98	178.75 ± 12.95
Butanolic fr.	324.70 ± 1.91	112.43 ± 1.87	405.09 ± 16.79
Ethyl acetate fr.	331.85 ± 2.40	178.93 ± 1.03	360.04 ± 16.19

CE showed the lowest amount of total phenolic content. Thus, the purification process increased the polyphenol-rich fractions with low impurities content

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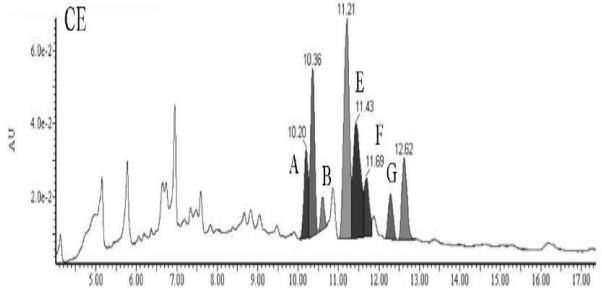
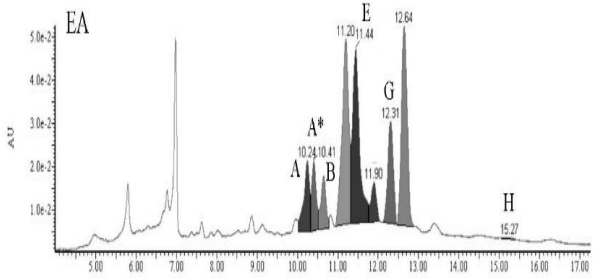
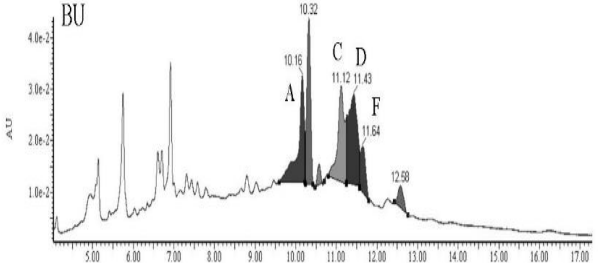
The fractions EA and BU presented a higher flavonoids and tannin content than the crude extract. However, the solubility of compounds is influenced by the solvent nature and their polarity. In case of EA, probably the existence of less polar substances in this extracts increased the flavonoid content.

CHARACTERIZATION

- Determination of phenolic compounds in the extracts and its fractions by HPLC technique with UV/Vis and MS detectors



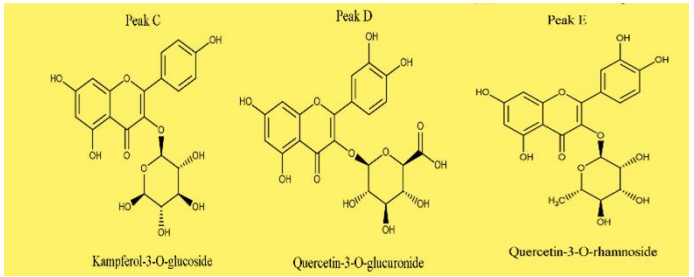




Phenolic compounds detected in *Ocotea lancifolia* leaf extract and corresponding fractions by LC-UV/ESI-MS, in the positive mode.

Proposed structure	Peak	Rt (min)	λ_{max} (nm)	MW	$[M+H]^+$ (m/z)	$[M+Na]^+$ (m/z)	Fragment ions (m/z)	% Estimated		
								CE	EA	BU
Quercetin-3-*O*-glucoside	A	10.16–10.24	256, 354	464	465	487	**303**	8.62	7.44	20.71
Quercetin-3-*O*-glucoside	A*	10.41	256, 354	464	465	487	**303**	–	5.72	–
Quercetin-3-*O* arabinoside	B	10.61–10.65	255, 354	434	ND	457	**303**, 312, 289	2.77	5.12	–
Kaempferol-3-*O*-glucoside	C	11.12	264, 351	448	449	471	**287**, 317	–	–	20.96
Quercetin-3-*O*-glucuronide	D	11.43	264, 350	478	ND	501	**303**	-	–	25.19
Quercetin-3-*O*-rhamnoside	E	11.43–11.44	256, 349	448	ND	471	**291**, **303**, 265	20.85	22.94	–
Isorhamnetin-3-*O*-glucoside 7-*O*-rhamnoside	F	11.64–11.70	254, 353	624	625	647	**317**, 291	7.01	–	7.71
Kaempferol-3-*O*-pentoside	G	12.30	264, 348	418	ND	441	**287**, 291	5.05	10.39	–
Catechin/Epicatechin	H	15.27	252	290	291	313	ND	–	0.35	–
Total identified								44.30	51.96	74.57

The base peaks are in bold; Rt: Retention time; A*: Diametric adduct; ND: not detected. CE: Crude extract; EA: Ethyl acetate fraction; BU: Butanolic fraction.

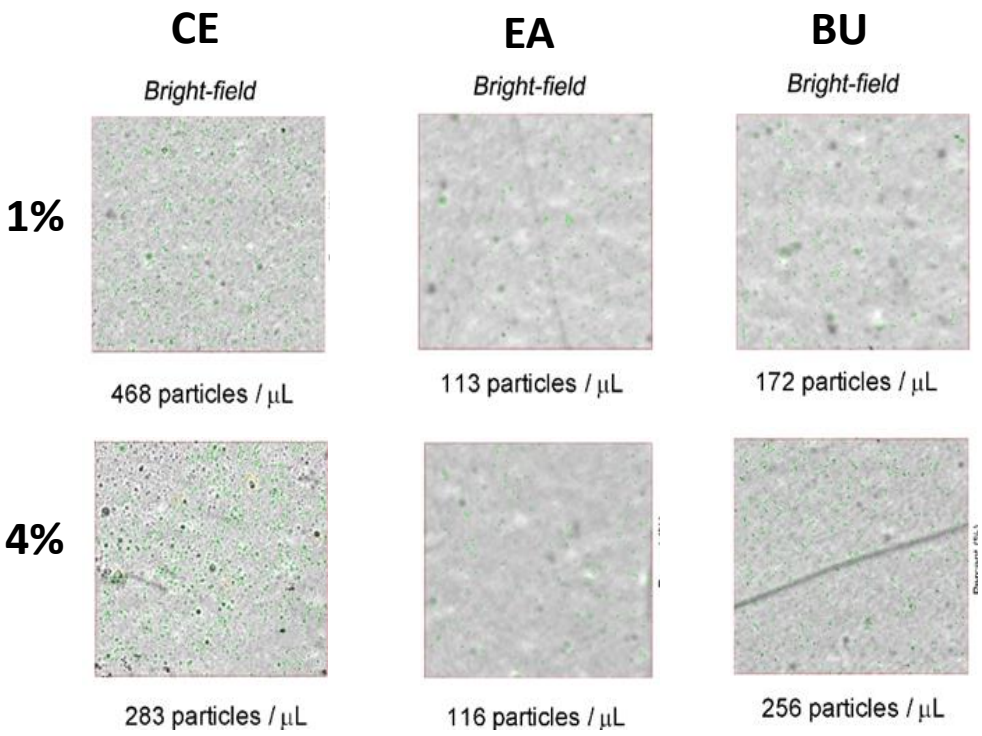


HPLC technique was used with UV/Vis and MS detectors to identify up to 74% of its composition, mainly flavonoid monoglucosides derived from quercetin were found in all the analyzed samples.

Ethyl acetate is a less polar solvent which presents the most appropriate polarity to solubilize less polar monoglucosides.

EXTRACTS SOLUBILITY

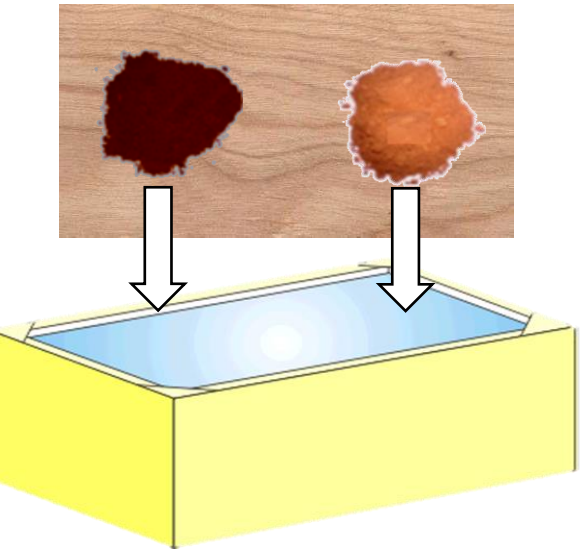
- The extracts solubility in organic solvents was tested with CE, EA and BU diluted in ethanol at 1% and 4% w/v.



The concentration of undissolved samples was analyzed using Cellometer Vision equipment.
Fractions present better solubility in ethanol than CE, with the higher solubility in EA.
Moreover, in the dissolutions at 1%, the number of particles was lower than in dissolutions at 4% being more soluble in ethanol at 1%.

WOOD VENEERS TREATMENT

- Uptake level of products on veneer samples.



Sample	Concentration (%)	Retentions (%)	
		Betula pubescens	Common beech
CE	1	1.43 ± 0.38	1.44 ± 0.25
	4	3.33 ± 0.99	3.30 ± 0.88
EA	1	1.20 ± 0.35	1.16 ± 0.20
	4	3.28 ± 1.05	3.57 ± 0.52
BU	1	1.06 ± 0.39	1.36 ± 0.33
	4	3.15 ± 0.54	2.72 ± 0.27

CE: Crude extract; EA: Ethyl acetate fraction; BU: Butanolic fraction.

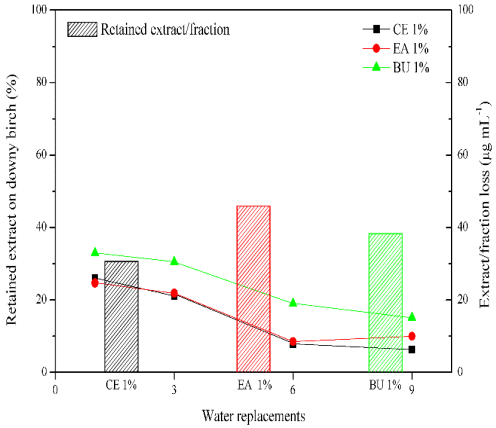
Veneers treated with crude extract or fractions at [1%] showed WPG of approximately 1.0-1.5%. Moreover, impregnation at [4%] showed values of WPG from 2.7 to 3.6%.
On the other hand, to achieve a successful product it is necessary to obtain a permanent high uptake of the tested samples, maybe by using additives that bond chemically veneer-extracts and to obtain a permanent penetration into the wood material.

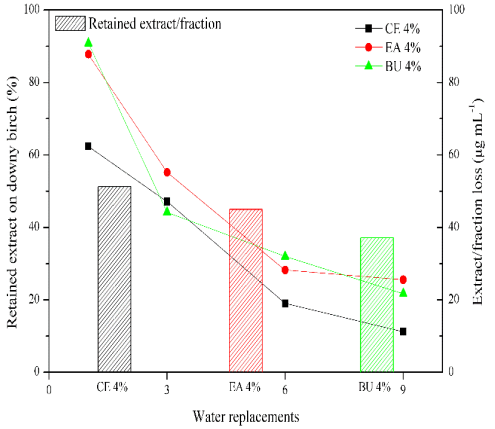
WOOD VENEERS TREATMENT

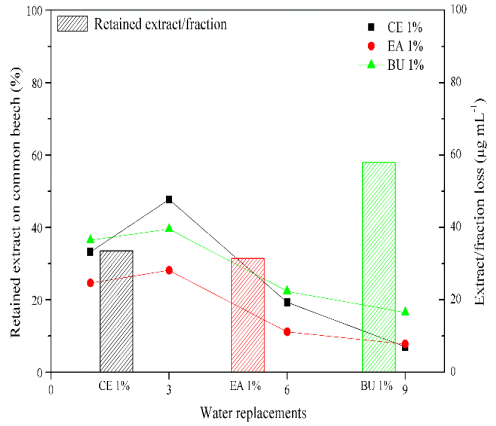
- Extracts fixation efficiency evaluated by leaching test

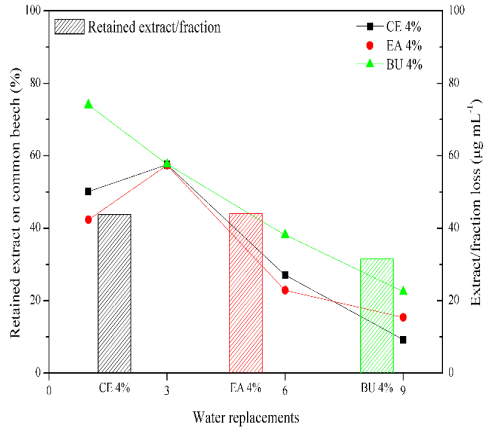
Leaching provides a fast approximation of changes during service life, and it as well could be used to measure the emission rates. Besides, it is an important step preceding the determination of the antifungal resistance.

The fixation of EA and BU were more efficient at [1%] (40-50%) compared with CE (approx. 30%), when using veneers of betula pubescens, Besides, BU was more efficient (57%) on beech veneers, and similar results were found EA and CE. At [4%] lower leaching rate on CE and EA was presented with both veneers, but not with BU in beech.







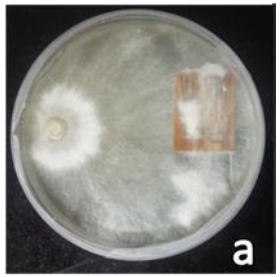


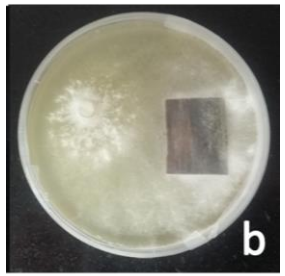
WOOD VENEERS TREATMENT

- Decay resistance test after 12 weeks

No.	Treatment	Mass loss (%)			
		G. trabeum		*T. versicolor*	
1	CE 1%	15.5 ± 5.1	ab	57.7 ± 24.1	b
2	CE 4%	6.6 ± 2. 7	a	3.1 ± 0.6	a
3	EA 1%	17.5 ± 3.7	b	1.8 ± 0.4	a
4	AE 4%	7.2 ± 5.2	a	2.3 ± 0.7	a
5	BU 1%	18.5 ± 5.8	b	68.7 ± 8.0	b
6	BU 4%	22.9 ± 3.8	b	55.4 ± 10.5	b
	Control	48.2 ± 9.3	c	48.1 ± 6.6	b

Data are reported as mean ± standard deviation. Different letters within the columns refer to means statistically different by Tukey test ($P < 0.05$). CE: Crude extract; EA: Ethyl acetate fraction; BU; Butanolic fraction.





CE 4%

AE 4%

BU 1%

Control veneers of *Betula pubescens* presented a mass loss of approx. 48%, indicating strong fungal activity under the test conditions. BU [4%] did not reduce mass loss of veneers impregnated with it, showing the lower antifungal effect, considering it as non-resistant to *T. versicolor* (ASTM D2017-81).

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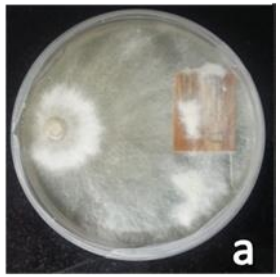

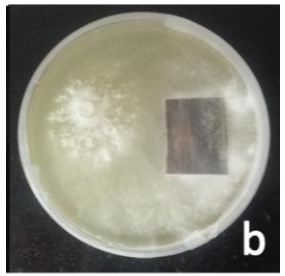

CE 4%

AE 4%

BU 1%

The highest antifungal effect was recorded on veneers treated with CE and EA at 4%; these values are considered as “highly resistance class” against wood-rot fungi. It is remarkable that the CE sample contain high amounts of sesquiterpenoids considers as antifungal agents.

SUMMARY

- The crude extract from *Ocotea lancifolia* leaves was obtained and separated into two fractions (butanolic and ethyl acetate fraction).
- The characterization showed higher phenolic content in the fractions than in crude extract, also it was possible to determine up to 74% of its composition.
- Despite impurities, the crude extract reduced the fungal attack on veneer of betula pubescens, as well as the ethyl acetate fraction at [4%].
- However, the butanolic fraction was not effective against wood-rot fungi.
- According to the results, O. lancifolia leaf extracts showed similar effectiveness compared with commercial plant-based preservatives, even at lower concentrations.
- **More info:** da Silva, D. T., Herrera, R., Batista, B. F., Heinzmann, B. M., & Labidi, J. (2017). Physicochemical characterization of leaf extracts from Ocotea lancifolia and its effect against wood-rot fungi. *International Biodeterioration & Biodegradation*, *117*, 158-170.

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COST FP1303
Performance of bio-based building materials

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Technical Workshop
"Design, Application and Aesthetics of biobased building materials"

Sofia, Bulgaria
28 February – 1 March, 2017

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Thank you for your attention!