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Propolis and organosilanes in wood protection. Part I: FTIR analysis and biological tests

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Abstract: *Propolis and organosilanes in wood protection. Part I: FTIR analysis and biological tests.* The paper presents the preliminary results of chemical and biological analyses of wood treated with a formulation based on ethanolic extract of propolis (EEP) and organosilicon compounds (methyltrimethoxysilane – MTMOS, vinyltrimethoxysilane – VTMOS). The results of biological test indicate the potential fungistatic properties of above mentioned formulation against *C. puteana*. The durability of the chemical bonds between wood and protecting system was confirmed by the chemical analysis using FTIR spectroscopy.

Keywords: propolis, Scots pine, FTIR, *Coniophora puteana*

INTRODUCTION

Propolis is a natural substance collected by honeybees from buds and leaf exudates of many deciduous trees (alder, poplar, birch) or coniferous trees (spruces, pine, fir), as well as shrubs and plants (Silici et al. 2007). The composition of propolis is highly complex and varies depending on the geographic origin and the collection season (Mavri et al. 2012). More than 300 chemical compounds have been identified so far from different propolis samples, including phenolic acids and their esters, flavonoids, amino acids, sugars or micro- and macroelements. Propolis shows pharmacological activities, such as antifungal, antibacterial, anticancer or anti-inflammatory to name a few (Silici et al. 2007; Mavri et al. 2012; Banksota et al. 2001). Fungicidal activity of propolis has been reported in numerous studies. The results of many research frequently indicate the activity of this bee product against clinically important microorganisms, e.g. *Candida albicans* (Silici et al. 2007; Mavri et al. 2012). Literature data shows that propolis inhibits the growth of *Penicillium italicum*, *Schizophyllum commune*, *Lenzites elegans* or *Ganoderma applanatum* (Quiroga et al. 2006; Yang et al. 2011). The Scots pine wood treated with a propolis extract showed activity against wood destroying fungi such as *Antrodia vaillantii, Gloeophyllum trabeum*, *Trametes versicolor*, *Coniophora puteana* and *Poria placenta* (Budija et al. 2008; Jones et al. 2011). The obtained results suggest that propolis may be used as a natural factor for bio-friendly wood protection.

Organosilicon compounds increase the resistance to biological attack and hydrophobicity of modified wood. Many wood properties, such as weather resistance, strength, fire resistance and dimensional stability, are improved by the application of organosilanes (Tingaut et al. 2006; Sebe, De Jeso 2000; Sebe, Brook 2001).

The aim of this study was to determine the properties of wood treated with a formulation containing propolis and organosilicon compounds (methyltrimethoxysilane – MTMOS, vinyltrimethoxysilane – VTMOS).

MATERIALS AND METHODS

Chemicals

The formulation used in this study consisted of 30% ethanolic extract of propolis, 8% MTMOS (methyltrimethoxysilane) $CH_3Si(OCH_3)_3$ (Sigma Aldrich) and 2% VTMOS (vinyltrimethoxysilane) $CH_2CHSi(OCH_3)_3$ (Sigma Aldrich).

Preparation of ethanolic propolis extract

Raw propolis was cut into small pieces and extracted with a 10-fold volume of 70% ethanol under shaking conditions. The extraction was carried out in the darkness and ambient temperature for 5 days. The final extract of propolis was concentrated on a rotary evaporator under the reduced pressure conditions at 40°C to constant weight. The final concentration (30%) of alcoholic extract was obtained by dissolution of suitable amount of residue in 70% ethanol.

FTIR analysis

Wood in the form of powder was mixed with KBr at a 1/200 mg ratio. Spectra were registered using the Infinity spectrophotometer by Mattson with the Fourier transform at a range of 500-4000 cm^{-1} at a resolution of 2 cm^{-1}, registering 64 scans.

Biological tests

The prepared formulation was used to treat the Scots pine (*Pinus sylvestris* L.) sapwood samples. The sample dimensions were as follows: 40×15×5 mm (the last dimension along the grain).Wood samples were impregnated by the vacuum and soaking methods and then subjected to mycological screening tests (modified EN 113) against the brown rot fungus *Coniophora puteana* (Schumacher ex Fries) Karsten (BAM Ebw. 15). The tests were conducted at 22 ± 1°C and the relative humidity of 70 ± 5%, and under the optimal conditions for the decay of lignocellulosic substance by *Basidiomycotina* fungi. Before exposure to fungi the samples were steam-sterilised in an autoclave (20 min, 121°C). The weight loss and the moisture content of wood samples were determined after 8 weeks of exposure.

Wood resistance to fungi was assessed according to the standard concerning classification of natural durability of wood (EN 350-1), by calculating the ratio (x = Ut/Uk) of average corrected mass loss of treated wood samples (Ut) to average mass loss of control samples (Uk).

RESULTS AND DISCUSSION

Figure 1 presents IR spectra of pine wood treated with 30% ethanolic extract of propolis (EEP) (B) and the preparation containing the propolis extract and MTMOS/VTMOS by vacuum (C) and soaking impregnation (D). Figure 1b presents a narrowed band at 2925 cm^{-1} (characteristic for the –OH bond) in IR spectra of wood treated with the preparation containing silanes. This confirms hydrophobic properties of this formulation.

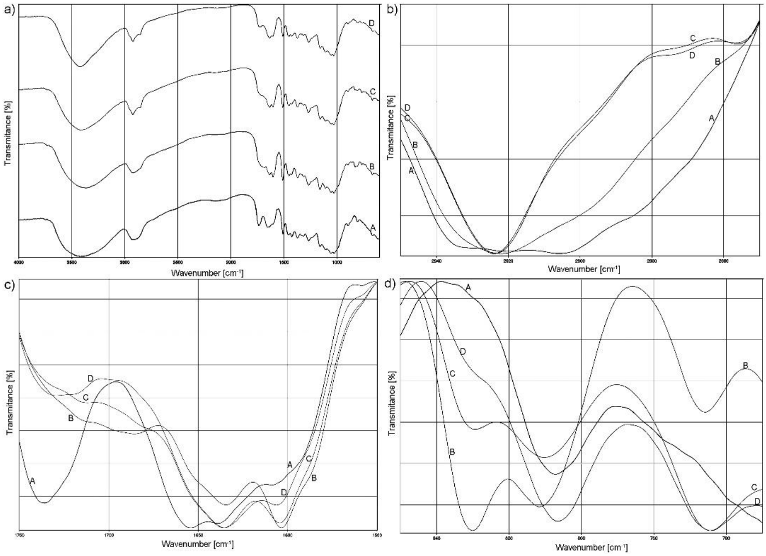

Figure 1. Spectra of untreated wood (A), treated wood: EEP VMI (B), EEP + VTMOS/MTMOS VMI (C), EEP + VTMOS/MTMOS SMI (D)
VMI – the vacuum impregnation method, SMI – the soaking impregnation method

A band at 1740 cm^{-1}, characteristic for the carbonyl bond of hemicelluloses (fig. 1c) can be also observed. The presence of this band indicates a chemical interaction of the propolis-silane preparation with wood. Moreover, in Fig. 1c we may see a band at 1605 cm^{-1}, most probably connected with the presence of unsaturated C=C bonds in the applied preparation. Additionally, the band in Fig. 1d at 830 cm^{-1}, characteristic for the Si–C and Si–O bands is also visible.

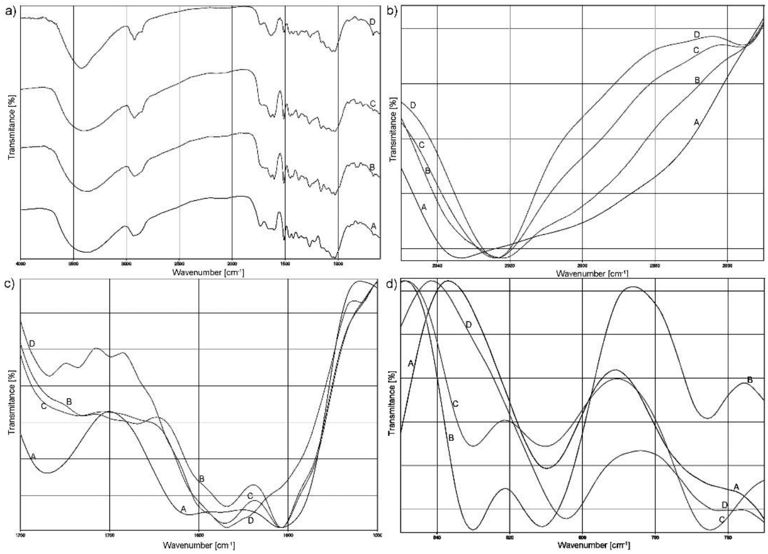

Figure 2. Spectra of wood after exposure to *C. puteana* (A), treated wood after exposure to *C. puteana*: EEP VMI (B), EEP + VTMOS/MTMOS VMI (C), EEP + VTMOS/MTMOS SMI (D)

Figure 2 presents the spectra of treated wood after its exposure to the fungus. No distinct changes were observed when comparing the spectra in Fig. 1a-d and Fig. 2a-d. This may indicate an effective fungicidal activity of the propolis-silane preparation, particularly in the case of vacuum impregnation.

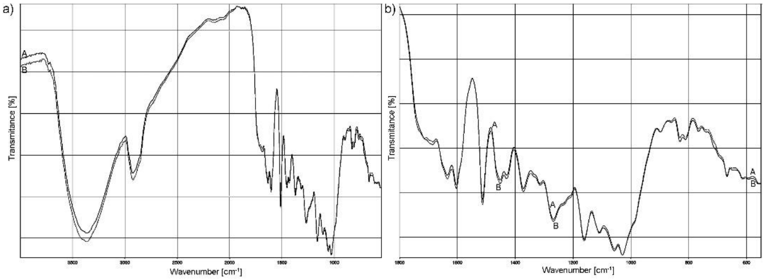

Figure 3. Spectra of wood treated 30% EEP VMI (A), wood treated EEP VMI after *C. puteana* (B)

Good fungicidal properties of the preparation are provided mainly by the propolis extract. The chemical evidence is given by the spectra presented in Fig. 3a-b. Figure 3 shows an IR spectrum of wood treated with the propolis extract (A) and that of a treated wood sample after the exposure to *C. puteana* (B). These spectra overlap (fig. 3b). This is an evidence that wood treated with propolis extract is resistant to fungus activity (no structural changes of wood can be seen after the exposure to *C. puteana*). The obtained results were confirmed by the spectra of untreated pine wood (A) and untreated pine wood exposed to *C. puteana* (B), presented in Fig. 4.

Interpretation of IR spectra were based on [Shang et al. 2013, Fackler, Schwanninger 2012, Pandey, Nagveni 2007, Irbe et al. 2011, Körner et al. 1992].

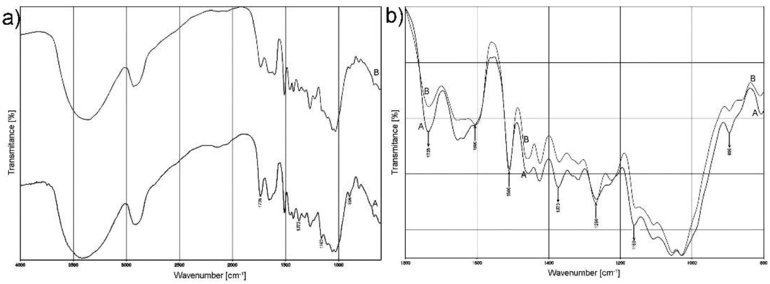

Figure 4. Spectra of wood sample (A), wood sample after *C. puteana* (B)

The intensity of the 1160 cm^{-1} band (C-O-C vibration in cellulose and hemicellulose) was reduced, which may indicate a decrease in the mean degree of polymerization of polycarbohydrates. The decrease in the intensity of the 895 cm^{-1} band (C-O-C stretching at β-1,4-glucoside linkages of cellulose and hemicellulose) also confirms the changes in the carbohydrate systems of wood. Thus, the assessment of changes at 1160 and 895 cm^{-1} may be applied in the evaluation of wood resistance against fungi. Moreover, in spectra presented in Fig. 4 at the simultaneous increase in relative absorption of the 1738, 1375, 1158, 898 cm^{-1} a decrease in the intensity of bands generated by lignin at 1600, 1505 and 1462 cm^{-1} was observed.

The exposure of pine wood to *C. puteana* resulted in low mass losses. The results of mycological test, presented in Table 1, indicate that wood treated with 30% ethanolic extract of propolis (EEP) and with formulation consisted of EEP and silicon compounds was quite resistant against examined fungi, in comparison with the untreated samples.

Table 1. The weight loss, wood moisture content and retention of wood after exposure to *C. puteana*

Sample		Retention	RSD	WMC [%]	RSD	WL [%]	RSD
EEP VMI	TS	216.6*	14.2	55.5	9.6	6.5	5.7
	CS	-	-	120.3	19.2	50.1	8.0
EEP + VTMOS + MTMOS VMI ***	TS	281.1*	15.8	23.6	2.0	3.7	2.2
	CS	-	-	57.3	5.4	43.8	0.7
EEP + VTMOS + MTMOS SMI ***	TS	83.7**	17.2	37.3	6.2	7.6	1.9
	CS	-	-	60.4	10.8	47.3	3.6

TS – tested sample, CS – control sample, WMC – wood moisture content, WL – weight loss,
* kg/m^3
** g/m^2,
*** (Mazela et al. 2015)
VMI – the vacuum impregnation method, SMI – the soaking impregnation method

The average weight loss of control samples was between 43-50%. The Scots pine wood samples treated with mixture of EEP and organosilanes exhibited a higher resistance against *C. puteana* than samples treated with a 30% extract of propolis alone. It is probably the effect of the silanes hydrolysis and the increased hydrophobic properties of modified wood. Moreover, wood samples impregnated by vacuum method exhibited better resistance against fungi than samples impregnated by soaking method. Compared to untreated Scots pine sapwood, which according to PN-EN 350-2 is classified as a class 5 – not durable as regards its resistance to *Basidiomycotina* fungi, pine wood treated with 30% extract of propolis and a mixture of propolis and organosilanes, impregnated by vacuum method, can be classified as a class 1 – very durable, while samples impregnated by soaking method can be classified as class 2 – durable.

REFERENCES

1. BANKSOTA A.H., TEZUKA Y., KADOTA S. 2001: Recent progress in pharmacological research of propolis. Phytother Res 15; 561-571
2. BUDIJA F., HUMAR M., KRICEJ B., METRIC M. 2008: Propolis for wood finishing. IRG/WP 08-30464
3. FACKLER K., SCHWANNINGER M. 2012: How spectroscopy and microspectroscopy of degraded wood contribute to understand fungal wood decay. Appl Microbiol Biotechnol 96: 587-599
4. IRBE I., ANDERSONE I., ANDERSONS B., NOLDT G., DIZHBITE T., KOURNOSOVA N., NUOPPONEN M., STEWART D. 2011: Characterisation of the initial degradation stage of scots pine (*Pinussylvestris* L.) sapwood after attack by brown-rot fungus *Coniophoraputeana*. Biodegradation 22: 719-728
5. JONES D., HOWARD N., WALES E. 2011: The potential of propolis and other naturally occurring products for preventing biological decay. IRG/WP 11-30575
6. KÖRNER I., FAIX O., WIENHAUS O. 1992: Attempt to determine brown-rot breakdown of Scots pine wood with the aid of FTIR spectroscopy. HolzalsRoh- und Werkst 50: 363-367

7. MAVRI A., ABRAMOVIC H.,POLAK T., BERTONCELJ J., JAMNIK P., MOZINA S.S., JERSEK B. 2012: Chemical properties and antioxidant and antimicrobial activities of Slovenian propolis. Chem Biodivers 9; 1545-1558
8. MAZELA B., BRODA M., PERDOCH W., GOBAKKEN L.R., RATAJCZAK I., COFTA G., KOMASA A., PRZYBYŁ A. 2015: Bio-friendly preservative systems for enhance wood durability – the first periodic report on DURAWOOD project. IRG/WP 15-30677
9. PANDEY K.K., NAGVENI H.C. 2007: Rapid characterisation of brown rot and white rot degraded pine and rubberwood by FTIR spectroscopy. HolzalsRoh- und Werkst 65: 477-481
10. QUIROGA E.N., SAMPIERTRO D.A., SOBERON J.R., SGARIGLIA M.A., VATTUONE M.A. 2006: Propolis from the northwest of Argentina as a source of antifungal principles. J Appl Microbiol 101; 103-110
11. SÈBE G., BROOK M.A., 2001: Hydrophobization of wood surfaces: covalent grafting of silicone polymers, Wood Sci. Tech. 35; 269-282
12. SÈBE G., DE JÉSO B., 2000: The dimensional stabilisation of maritime pine sapwood (*Pinuspinaster*) by chemical reaction with organosilicon compounds. Holzforschung 54; 474-480
13. SHANG J., YAN S., WANG Q. 2013: Degradation mechanism and chemical component changes in *Betulaplatyphylla* wood by wood-rot fungi, BioResources 8 (4): 6066-6077
14. SILICI S., UNLU M., VARDAR-UNLU G. 2007: Antibacterial activity and phytochemical evidence for the plant origin of Turkish propolis from different regions. World J Micro Biot 23; 1797-1803
15. TINGAUT P., WIEGENAND O., MAI C., MILLITZ H., SÈBE G., 2006: Chemical reaction of alkoxysilane molecules in wood modified with silanol groups. Holzforschung 60; 271-277
16. YANG S.Z., PENG L.T., SU X.J., CHEN F., CHENG Y.J., FAN G., PAN S.Y. 2011: Bioassay-guided isolation and identification of antifungal components from propolis against *Penicilliumitalicum*. Food Chem 127; 210-215

Streszczenie: *Propolis i organosilany w ochronie drewna. Część I: analiza FTIR i testy biologiczne.* W pracy zbadano oddziaływanie etanolowego ekstraktu propolisu oraz jego mieszaniny z organosilanami – VTMOS (winylotrimetoksysilan) i MTMOS (metylotrimetoksysilan) na drewno sosny zwyczajnej (*Pinus sylvestris* L.). Analizę strukturalną drewna impregnowanego etanolowym ekstraktem propolisu (EEP) i jego mieszaniną ze związkami krzemoorganicznymi wykonano metodą spektroskopii w podczerwieni (FTIR). Przedstawione wyniki FTIR drewna mogą świadczyć o chemicznym oddziaływaniu badanych preparatów impregnacyjnych z substancją drzewną. Ponadto wyniki testów mykologicznych wskazują, na właściwości fungistatyczne stosowanych preparatów.

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