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The Possibility of Propolis Extract Application in Wood Protection

Magdalena Woźniak [1,*], Patrycja Kwaśniewska-Sip [2,3], Agnieszka Waśkiewicz [1], Grzegorz Cofta [3] and Izabela Ratajczak [1]

[1] Department of Chemistry, Faculty of Wood Technology, Poznań University of Life Sciences, Wojska Polskiego 75, 60625 Poznań, Poland; agnieszka.waskiewicz@up.poznan.pl (A.W.); izabela.ratajczak@up.poznan.pl (I.R.)
[2] Air Quality Investigation Department, Łukasiewicz Research Network-Wood Technology Institute, Winiarska 1, 60654 Poznań, Poland; p_kwasniewska@itd.poznan.pl
[3] Institute of Chemical Wood Technology, Faculty of Wood Technology, Poznań University of Life Sciences, Wojska Polskiego 38/42, 60637 Poznań, Poland; grzegorz.cofta@up.poznan.pl
* Correspondence: magdalena.wozniak@up.poznan.pl; Tel.: +48-61-848-78-38

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Abstract: Nowadays, there is a growing interest in extending the service life of wood and wood products by applying natural substances that are harmless to humans and the environment. In this paper, propolis was used as an eco-friendly wood preservative. The aim of this study was to determine the resistance of Scots pine wood treated with the propolis extract against brown-rot fungus *Coniophora puteana*. The wood biodegradation was assessed by gravimetric method, as well as by the analysis of ergosterol concentration in decayed wood and by the determination of changes in the wood structure by means of Fourier transform infrared spectroscopy. The results indicated that the impregnation of wood with propolis extract above 12% concentration limited fungal decay. The mass loss of wood treated with 18.9% propolis extract was 2.3% and was over 21 times lower than that for untreated wood. The analysis of ergosterol content and the changes in wood structure also confirmed that the propolis extract above 12% concentration protected wood against decay caused by *C. puteana*. Moreover, the propolis extract used in wood impregnation was rich in phenolic compounds, mainly chrysin, pinocembrin and galangin, which possess antimicrobial activity. The obtained results indicate that the extract of Polish propolis can be a promising natural wood preservative, safe for humans and the natural environment.

Keywords: propolis; Scots pine; *Coniophora puteana*; ergosterol; natural preservatives

1. Introduction

Nowadays, there is a growing interest in extending the service life of wood and wood products using environmentally friendly preservatives. These eco-friendly wood protection agents are often based on natural substances and other chemical compounds with low or no toxicity for humans and minimum environmental impact.

In recent years, the interest in the use of natural substances in wood protection has been growing in the literature. Essential oils from various plants as biocides in wood treatment are widely investigated worldwide. Thyme, oregano, clove, sweet flag, lemon grass and lavender oils were the most effective oils for wood protection against both mold and decay fungi [1–3]. Moreover, the constituents of essential oils were tested as bioactive compounds in wood protection. Eugenol, cinnamaldehyde and isoeugenol showed activity against wood decay fungi [3–5]. Natural substances with potential application in wood protection also include: chitosan, caffeine, waxes, resins and natural oils, such as

linseed, tung and tall oil [3,6–11]. An interesting natural substance that has also been of interest as an antifungal agent in the protection of wood is propolis [12,13].

Propolis is a resinous material collected by honeybees (*Apis mellifera*) from various tree buds and plant exudates [14,15]. Its chemical composition is complex and depends on the plant sources available to the bees in different geographical regions. Propolis contains various chemical compounds, such as phenols (flavonoids, phenolic acids and their esters), terpenoids, fatty acids, sugars, as well as mineral elements [16–19]. Phenolic compounds are biologically active components of propolis, which are mainly responsible for the pharmacological activity of propolis extracts [15,20]. Propolis extracts exhibit antioxidant, antiviral, antibacterial, antifungal, antiproliferative and hepatoprotective activity [14,19,21–26]. The antifungal property of propolis extract was confirmed in many literature data, which described its activity against pathogenic fungi (*Penicillium italicum*, *Aspergillus niger*, *Trichoderma viride*, and *Colletotrichum gloeosporioides*), as well as fungi causing wood decay (*Ganoderma applanatum*, and *Pycnoporus sanguineus*) [27–30]. Propolis extract was also used in wood protection as an agent increasing the natural durability of wood against fungi. Budija et al. [12] indicated that spruce wood treated with 29% propolis extract showed resistance against *Trametes versicolor*, *Antrodia vaillantii,* and *Gloeophyllum trabeum*, in comparison to untreated wood. The impregnation of pine wood with a soda-based propolis solution limited the growth of *Poria placenta* and caused the resistance of treated wood against *C. puteana* [31]. In turn, Scots pine and paulownia wood treated with Turkish propolis extracts exposed to *T. versicolor* and *Neolentinus lepideus* showed a lower mass loss than that of untreated wood [13]. Propolis was also used as a constituent of the preparation containing chitosan, propolis extract and silver nanoparticles in preventing the decay of wood caused by *T. versicolor* [32,33]. The preparation based on propolis extract, caffeine and organosilanes was tested in protection of wood against brown-rot fungus *C. puteana* [34]. In addition, according to the literature data, wood treated with the propolis extract with silicon compounds showed an increased resistance against *C. puteana* and improved hydrophobic properties [35,36].

The aim of this study was to determine the antifungal activity of wood treated with the extract of Polish propolis against the brown-rot fungus *C. puteana*. The wood biodegradation was assessed by gravimetric method, as well as by the analysis of ergosterol concentration in decayed wood. Moreover, Fourier transform infrared spectroscopy was used to determine the changes in the structure of wood exposure to fungus.

2. Materials and Methods

2.1. Wood Samples

The investigated material was Scots pine (*Pinus sylvestris* L.) sapwood with a dimension of $5 \times 10 \times 40$ mm^3 (the last dimension along the fibers). All wood specimens were prepared from several logs supplied by the Faculty of Wood Technology from Poznań University of Life Sciences. The tested samples were without knots or other growth inhomogeneity, and their average density was 0.540 g/cm^3.

2.2. Propolis Extract

The 30% ethanolic extract of Polish propolis was purchased form PROP-MAD—a company producing propolis extract from Poznań in Poland. The extract was prepared in 70% ethanol. For wood impregnation, the propolis extract in the initial concentration was diluted according to the Annex A Calculation of correction factors for organic wood preservative, which is a part of EN 113:1996 (100%, 63%, 40%, 25% and 10%) [37]. The final concentrations of the propolis extract were: 30.0%; 18.9%; 12.0%; 7.5% and 3.0%. For the dilution of propolis extract 70% ethanol was used.

2.3. Wood Treatment

The wood samples were dried at 103 °C for 24 h to determine dry matter. The wood (5 samples per treatment) was treated with propolis extract at 5 different concentrations by the vacuum method—15 min

under vacuum conditions—0.8 kPa and 2 h under an atmospheric pressure, according to EN 113:1996 [37]. After impregnation, all the samples were immediately removed from the extract and weighed to determine the propolis extract uptake. The wood sample retention (kg/m^3) was calculated by following equation:

$$R\left(kg/m^3\right) = \frac{(M_b - M_a)\cdot c\cdot 10}{v} \quad (1)$$

where: M_a—the wood mass before treatment (g); M_b—the wood mass after treatment (g); c—the concentration of the propolis extract (%); v—volume of the wood sample (m^3).

After impregnation, all the wood samples were cured for four weeks in room conditions (RH = 65 ± 5%; T = 20 ± 2 °C).

2.4. Decay Resistance Test

The decay resistance of the wood treated with the propolis extract against brown-rot fungus—*Coniophora puteana* (Schumacher ex Fries) Karsten BAM 112 (BAM Ebw. 15)—was carried out in accordance with the modified EN 113:1996 [37]. The agar medium consisting of malt extract powder (50 g), agar (20 g) and deionized water (1000 mL) was used to grow the fungi cultures. The wood samples were placed into Petri dishes and exposed to decay fungi at 22 °C ± 2 °C and a relative humidity of 70% ± 5% for 8 weeks. After this time, the fungus mycelium was removed from each wood sample and the samples were weighed. The decay resistance was determined according to the percentage of the mass loss of the wood samples caused by the fungi, which was calculated based on the mass difference before and after the test.

2.5. Ergosterol Concentration Analysis

The ground wood samples after exposure to *C. puteana* (0.1 g) were extracted with 2 mL methanol and 0.5 mL of 2M aqueous sodium hydroxide (Avantor Performance Materials, Gliwice, Poland). The samples were irradiated thrice in a microwave oven (370 W) for 10 s. After cooling down, the solutions were neutralized with 1M aqueous hydrochloric acid (Avantor Performance Materials, Gliwice, Poland). Then, the samples were thrice extracted with 4 mL of *n*-pentane (Sigma Aldrich, Darmstadt, Germany). The combined pentane extracts were evaporated for dryness and before analysis dissolved in 1 mL of methanol and 20 µL of the thus prepared mixture was analyzed by high pressure liquid chromatography (HPLC) using the Waters 2695 chromatographer (Waters, Manchester, MA, USA). The ergosterol separation was performed on a 3.9 mm Nova Pak C-18, 4 mm column, with methanol: acetonitrile (90:10, v/v) as the mobile phase, at a flow rate of 1.0 mL/ min. Ergosterol was detected with a Waters 2996 Photodiode Array Detector (Waters, Manchester, MA, USA) set at 282 nm. The presence of ergosterol was confirmed by a comparison of the retention times with the external standard and by co-injection of every tenth sample with an ergosterol standard. The detection limit was 0.01 µg/g and standard deviation was below 7%. Each sample was made in triplicate and three independent experiments were performed.

2.6. Fourier Transform Infrared Spectroscopy (FTIR)

The ground wood samples were mixed with KBr (Sigma Aldrich, Darmstadt, Germany) at a 1/200 mg ratio and in the form of a pellet were analyzed using the Nicolet iS5 spectrophotometer with Fourier transform (Thermo Fisher Scientific, Waltham, MA, USA). The spectra were registered, at a range of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}, registering 16 scans.

2.7. Identification of Phenolic Compounds in Propolis Extract

The concentration of the selected phenolic compounds in the propolis extract was determined by a UPLC–PDA–TQD system. The chromatographic system consisted of the Aquity UPLC chromatograph

(Waters, Manchester, MA, USA) equipped with a photodiode detector (PDA el Detector) (Waters, Manchester, MA, USA) and coupled to an electrospray ionization triple quadrupole mass spectrometer (TQD) (Waters, Manchester, MA, USA). All the standards of phenolic compounds (apigenin, chrysin, epicatechin, catechin, galangin, kaempferol, myricetin, genistein, naringenin, pinobanksin, pinocembrin, pinostrobin, quercetin, rutin, caffeic acid, coumaric acid, ferulic acid, cinnamic acid, vanillic acid, chlorogenic acid, hydroxybenzoic acid, sinapic acid and syringic acid) were purchased from Sigma Aldrich (Darmstadt, Germany). The propolis extract was evaporated to dry mass and the residue was diluted in methanol of chromatographic grade (Sigma Aldrich, Darmstadt, Germany) and filtered through a 0.20 µm syringe filter (Chromafil, Macherey-Nagel, Duren, Germany). The tested compounds were separated at 25 °C on the analytical column—a Waters ACQUITY UPLC HSS T3 (150 × 2.1 mm/ID, with 1.8 µm particle size) (Waters, Manchester, MA, USA). The gradient elution was applied using water containing 0.1% HCOOH (A) and acetonitrile containing 0.1% HCOOH (B) with the flow rate at 300 µl/min. The solvent gradient was modified as follows: 0–5 min 25% B, 5–20 min 40% B, 20–30 min 60% B, 30–35 min 90% B, 35–40 min 100% B followed by the return to the initial conditions. The analytes were identified by comparing the retention times and the m/z values obtained by MS and MS^2 with the mass spectra of the corresponding standards tested under the same conditions. The sample of propolis extract was injected in triplicate.

2.8. Statistical Analysis

The results were analyzed using a one-way analysis of variance (ANOVA) applying Tukey's Honest Significant Differences (THSD) Test. Statistical significance was defined as $p < 0.05$. All the statistical analyses were performed using the TIBCO Software Inc. Statistica version 13 (Palo Alto, CA, USA).

3. Results and Discussion

3.1. Decay Test

In the first stage of the research process, the activity of the Scots pine wood treated with the ethanolic propolis extract in the range of 3–30% concentration against *C. puteana* was determined. The choice of solvent (70% ethanol) for the propolis extraction was based on our previous studies, which analyzed the effect of the solvent used for the extraction of propolis on its antifungal activity. Based on the results of the propolis extraction yield, antifungal activity and the ecological aspect, 70% ethanol was chosen as the solvent for propolis extraction [38]. In turn, the maximum concentration of propolis extract (30%) used for wood treatment was selected on the basis of the literature data and taking into account the economic aspect [12]. The results of the antifungal efficacy against *C. puteana*, expressed as average mass loss of wood samples treated with the propolis extract in various concentrations, are presented in Table 1.

Table 1. Retention and mass loss of treated wood after exposure to *C. puteana*.

Propolis Extract Concentration (%)	Retention (kg/m^3)	Mass Loss (%)
0	-	48.8 ^{a} ± 1.1
3.0	19.0 ± 0.3	31.6 ^{b} ± 1.6
7.5	48.0 ± 0.5	5.9 ^{c} ± 0.7
12.0	80.3 ± 0.3	3.3 ^{d} ± 0.4
18.9	129.2 ± 0.4	2.3 ^{d} ± 0.3
30.0	216.6 ± 0.5	2.7 ^{d} ± 0.5

Expressed as average ± standard deviations. Values denoted with identical letters do not differ significantly.

The results indicated that the action of fungus caused the mass loss of wood samples treated with the propolis extract in the range of 2.3% to 31.6%. In turn, the mass loss of untreated control samples was 48.8%. The protective activity of the propolis extract can be seen in the comparison of the mass loss between the treated and untreated wood samples. The antifungal behavior of the treated wood

was observed even at a low concentration of the propolis extract. The pine wood with a treatment retention of 129.2 kg/m^3 of the propolis extract exhibited the lowest value of mass loss (2.3%). The wood impregnated with 18.9% propolis extract was over 21 times more resistant against the tested fungus than the untreated samples. The low value of mass loss was also observed for wood treated with 30% propolis extract. The wood samples protected with 18.9% and 30% propolis extract after exposure to *C. puteana* are presented in Figure 1. The increase in the concentration of the propolis extract resulted in a more effective protection of wood against decay fungus. Statistical analysis indicated that the differences in the mass loss of the wood treated with the propolis extract at the retention threshold of 80 to 216 kg/m^3 did not differ significantly. The results of the decay test suggest that the propolis extract with a concentration above 12% can protect wood against fungal decay caused by *C. puteana*.

(**a**) (**b**)

Figure 1. Scots pine wood after exposure to *C. puteana*: (**a**) control wood sample and wood treated with 18.9% propolis extract; (**b**) control wood sample and wood treated with 30% propolis extract.

According to the literature data, various species of wood treated with propolis extracts exhibited resistance against wood-decay fungi, including *G. trabeum*, *T. versicolor*, *C. puteana*, *P. placenta*, and *N. lepideus* [12,13,31]. The mass loss of pine wood treated with 7% propolis extract and exposed to *T. versicolor* and *N. lepideus* was 4.2% and 2.5%, respectively [13]. In turn, poplar wood protected with propolis extract in concentrations of 5-40 mg/mL after four weeks of exposure to *T. versicolor* showed the mass loss in the range of 13.7–9.8%, compared to the 14% mass loss of the unprotected wood [32]. The weight loss of the wood treated with chitosan oligomers and propolis after 30 days of exposure to *T. versicolor* was 32.8% compared to 42.3% of the weight loss of the untreated wood [33]. The pine wood impregnated with the preparation consisting of propolis extract, caffeine and organosilanes (methyltrimethoxysilane and octyltrimethoxysilane) was five times more resistant against the activity of *C. puteana* than the unprotected samples [34]. The earlier work of authors indicated that pine wood treated with the extract of Polish propolis with silicon compounds was more durable against *C. puteana* than untreated wood [36,39]. The mass loss of wood treated with 30% propolis extract by soaking was 6.5%, while the mass loss of wood treated with 30% propolis extract by vacuum method was 2.7%, suggesting that the vacuum method was a more effective method for wood impregnation with propolis extract [36].

3.2. Ergosterol Concentration

Fungal activity in wood can be determined by measuring the weight loss of the wood after exposure to test fungi, but the level of fungal attack in wood can also be assessed by analyzing the ergosterol content in the infected material [40]. The ergosterol concentration and the percentage reduction of ergosterol in treated wood samples in relation to the ergosterol content in untreated wood are presented in Table 2.

Table 2. Ergosterol concentration and the ergosterol reduction in wood after exposure to *C. puteana*.

Propolis Extract Concentration (%)	Ergosterol Concentration (μg/g)	Ergosterol Reduction (%)
0	180.00 [a] ± 5.58	-
3.0	118.66 [b] ± 6.49	34
7.5	100.93 [c] ± 1.12	44
12.0	85.68 [d] ± 2.71	52
18.9	57.92 [e] ± 2.63	68
30.0	28.96 [f] ± 1.03	84

Expressed as average ± standard deviations. Values denoted with identical letters do not differ significantly.

The ergosterol concentration determined in wood samples exposed to *C. puteana* indicated that the propolis extract limited the growth of fungal mycelium. The decrease in fungal activity in the treated wood samples was associated with the concentration of propolis extract used for impregnation. The value of ergosterol reduction in the treated wood increased with the increase of propolis concentration from 34% (3% propolis extract) to 84% (30% propolis extract). A significant decrease in the ergosterol content in wood (from 180.27 to 85.68 μg/g) was already observed in wood treated with 12% propolis extract. The results of the ergosterol analysis were also consistent with the results of the weight loss of infected wood samples (mainly in the range of propolis concentration 3.0–18.9%). The value of the ergosterol reduction increased with the decrease in the value of the mass loss of treated wood. In addition, in the literature data the ergosterol concentration in treated wood samples was analyzed to identify mycelium in wood. Perdoch et al. [41] showed that in most cases of wood treated with preservatives based on IBPC (3-iodo-2-propynyl butylcarbamate) and silicon compounds and exposed to *C. puteana*, the ergosterol content was consistent with the mass loss of the wood samples.

3.3. FTIR Analysis

The changes in the pine wood treated with the propolis extract decayed by *C. puteana* were determined by FTIR (Figure 2b–f). The FTIR spectra of the untreated wood before and after exposure to brown-rot fungus are presented in Figure 2a.

The changes in the FTIR spectrum of the wood following the action of brown-rot fungi, including *C. puteana*, which selectively decay carbohydrates and limit lignin degradation, have been described in the literature [34,42–47]. The most important changes in the spectrum of wood exposure to *C. puteana* were observed in the fingerprint region, between 1800 and 600 cm^{-1} and were characterized on the basis of the literature data [13,33,42–47]. The well defined bands in the FTIR spectra of the untreated and undecayed wood were observed at: 1735 cm^{-1} for unconjugated C=O from xylans in hemicellulose, 1600 cm^{-1} for aromatic skeletal and C=O stretch vibration in lignin, 1505 cm^{-1} for C=C aromatic skeletal vibration in lignin, 1370 cm^{-1} for C-H bond in cellulose and hemicellulose, 1321 cm^{-1} for C-O in syringyl and guaiacyl rings and O-H in plane bending in cellulose, 1262 cm^{-1} guaiacyl ring breathing, 1160 cm^{-1} for C-O-C vibration in cellulose and hemicellulose and 897 cm^{-1} for C-O stretch in cellulose and hemicellulose. The intensity of carbohydrates bands (1735, 1370, 1320, 1160 and 897 cm^{-1}) in the spectrum of wood after exposure to *C. puteana* decreased in comparison to the spectrum of undecayed wood. In turn, the intensity of the transmittance bands at 1600, 1505 and 1262 cm^{-1} increased in the spectrum of decayed wood compared to the spectra of the undecayed wood. These changes in the FTIR spectra were connected with an increase in lignin content relative to carbohydrate evident in wood degraded by *C. puteana*.

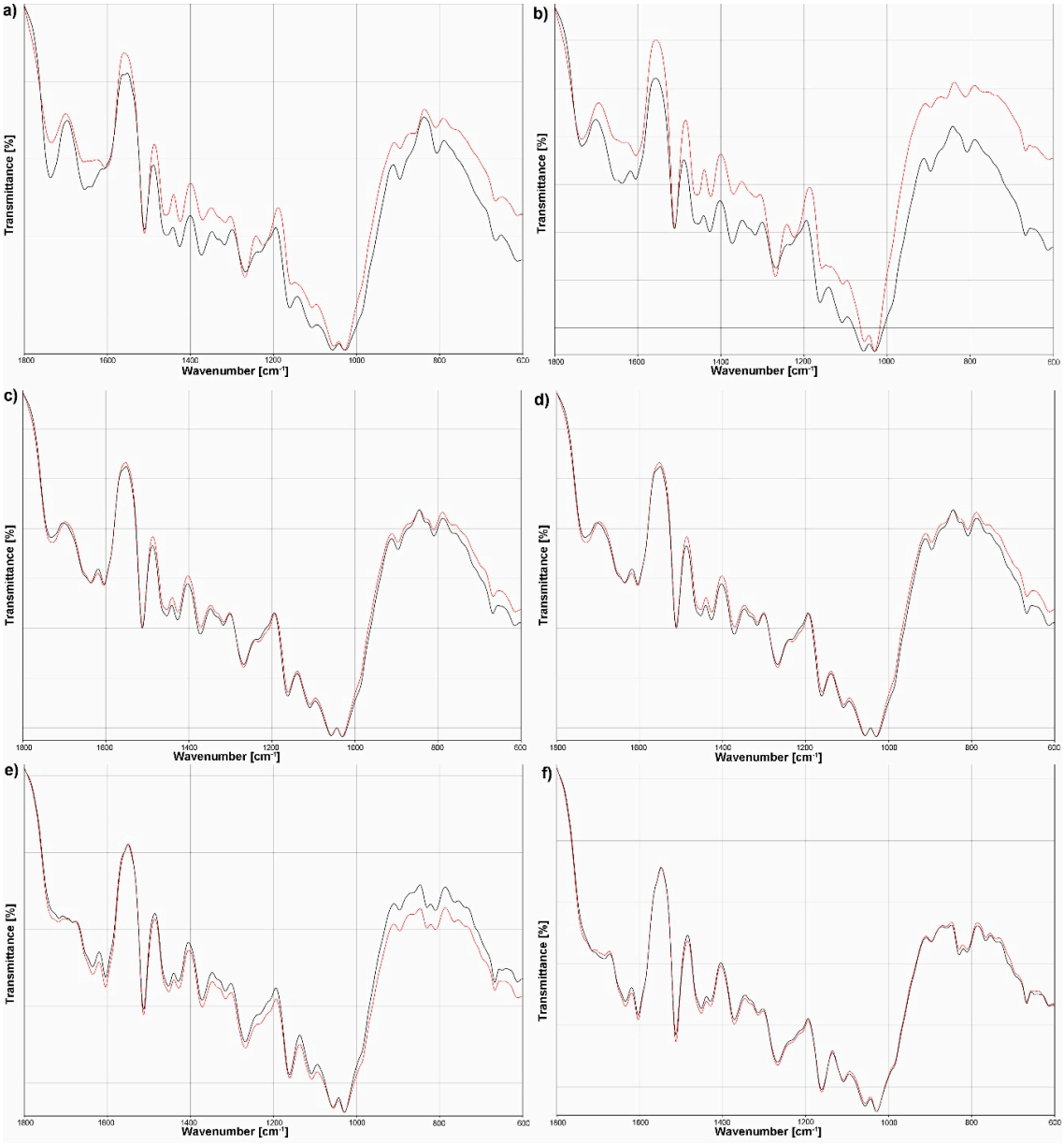

Figure 2. The Fourier Transform Infrared Spectroscopy (FTIR) spectra of the Scots pine wood before the exposure to *C. puteana* (—) and after the exposure to *C. puteana* (—): (**a**) untreated wood (control wood sample), (**b**) wood treated with the 3% propolis extract, (**c**) wood treated with the 7.5% propolis extract, (**d**) wood treated with the 12% propolis extract, (**e**) wood treated with the 18.9% propolis extract and (**f**) wood treated with the 30% propolis extract.

In the spectrum (Figure 2b) of the wood treated with 3% propolis extract after exposure to the tested fungus, a decrease in the intensity of the polysaccharide bands at 1735, 1370, 1320, 1160 and 897 cm^{-1}, and an increase in the relative intensity of the bands assigned to lignin at 1600 and 1262 cm^{-1}, compared to the spectrum of the undecayed treated wood, were observed. The observed changes in the FTIR spectra of the wood treated with 3% propolis extract were consistent with the results of mass loss and ergosterol content, suggesting that 3% propolis extract did not completely protect the wood against the decay fungus. In the spectra of the wood treated with a higher concentration of the propolis extract, no significant changes were observed, as in the spectra of the untreated wood or treated with 3% propolis extract with exposure to *C. puteana*. In the spectrum (Figure 2c) of the wood treated with 7.5% propolis extract with exposure to decay fungus the intensity of the bands at

1375 and 897 cm^{-1} was lower than in the spectrum of the treated wood before the action of *C. puteana*. In the spectrum (Figure 2d) of the wood treated with 12% propolis extract with exposure to the tested fungus, a decrease in the band intensity was observed at 1375 cm^{-1} compared to the spectrum of the treated wood without exposure to the fungus. In the spectrum (Figure 2e) of the wood impregnated with 18.9% propolis extract with exposure to the fungus, the intensity of band at 1600 cm^{-1} increased and the intensity of band at 897 cm^{-1} decreased compared to the spectrum of the treated wood before exposure to *C. puteana*. In turn, in the spectra (Figure 2f) of the wood treated with 30% propolis extract before and after exposure to the tested fungus, the intensities of all the mentioned bands were very similar, suggesting that the propolis extract at this concentration protected the pine wood from the destructive effect of *C. puteana*.

3.4. Analysis of Propolis Extract Composition

According to the available literature, the biological activity of propolis is associated with the presence of bioactive components in which the phenolic compounds are mainly responsible for the pharmaceutical effect of European propolis [15,20]. Therefore, the concentrations of selected flavonoids and phenolic acids were determined in the propolis extract used in this study. The concentrations of the phenolic compounds in the propolis extract are shown in Table 3.

Table 3. Concentrations of the flavonoids and the phenolic acids in the propolis extract.

Phenolic Compound	Concentration (mg/g of Extract)
Apigenin	11.98 ± 0.49
Chrysin	23.33 ± 0.69
Galangin	28.96 ± 0.58
Kaempferol	16.33 ± 0.84
Quercetin	3.23 ± 0.25
Myricetin	0.80 ± 0.08
Naringenin	1.14 ± 0.18
Pinobanksin	3.85 ± 0.26
Pinocembrin	45.68 ± 0.78
Rutin	0.37 ± 0.02
Caffeic acid	3.08 ± 0.43
Coumaric acid	10.34 ± 0.66
Ferulic acid	2.74 ± 0.28
Cinnamic acid	6.54 ± 0.61
Chlorogenic acid	0.26 ± 0.05
Vanillic acid	0.10 ± 0.04
Hydroxybenzoic acid	0.34 ± 0.70

Expressed as average ± standard deviations.

The results of the quantitative analysis of flavonoids in the propolis extract showed that pinocembrin was detected in the largest amount. High concentrations in the extract were also recorded for chrysin and galangin. Pinocembrin, galangin and chrysin are flavonoids that are commonly identified in propolis samples from various parts of Poland [19,24]. These compounds were also identified in propolis samples collected from Italy, China, Argentina, Greece or Macedonia [48–50]. Moreover, these flavonoids were indicated as possible compounds responsible for the antifungal activity of propolis [20,27,30]. The antimicrobial activity of propolis was also attributed to caffeic acid, which was identified in the tested extract [17]. Among phenolic acids, coumaric acid was found in the largest amount in the propolis extract. A high level of concentration was also detected for cinnamic acid. All identified aromatic acids in the propolis extract were previously detected in propolis samples collected from Poland [18,19,24]. The concentrations of catechin, epicatechin, genistein, pinostrobin, synaptic acid and syringic acid in the propolis extract were below the UPLC/PDA/TQD detection limits.

4. Conclusions

This study assessed the possibility of using the propolis extract as a wood preservative against brown-rot fungus *C. puteana*. The results of the decay test showed that the propolis extract limited the fungus activity even at low concentration. The most effective protection against *C. puteana*, expressed as a mass loss, was observed for samples with propolis extract retention ranging from 80 to 216 kg/m^3. The mass loss of the wood treated with 18.9% propolis extract was 2.3% and was over 21 times lower than that for the untreated control samples. The antifungal activity of the propolis extract used for the wood treatment was also confirmed by analyzing the ergosterol content in decayed wood samples. The decrease in fungal activity in the treated wood was associated with the increasing concentration of the propolis extract used for impregnation. The effects of the brown-rot fungus on the wood treated with the propolis extract was also assessed, analyzing the changes in its structure. The intensity changes in the FTIR spectra of the wood following the action of the brown-rot fungus, mainly at 1735, 1600, 1375 and 897 cm^{-1}, were less significant in the spectra of the wood treated with the propolis extract at higher concentrations (12–30%) compared to the spectra of the untreated wood or wood treated with 3% propolis extract. All three methods of analyzing the fungal resistance of the wood treated with the propolis extract were complementary and clearly indicated that the extract of Polish propolis protected the Scots pine wood against *C. puteana*. Moreover, the propolis extract contained a high concentration of phenolic compounds, mainly pinocembrin, galangin and chrysin, which possess antimicrobial activity.

In summary, based on the results of the research, it can be stated that propolis, due to its antifungal properties and natural origin, could be a promising natural wood preservative safe for humans and the environment. Propolis extracts, due to absence of a large amount of organic volatile solvents, could be used for the impregnation of wooden elements in internal applications. In further studies, it seems advisable to determine the applicability of propolis extract for wood protection in outdoor applications.

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