Pyrolysis distillates from tree bark and fibre hemp inhibit the growth of wood-decaying fungi

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Abstract

The quest for cleaner wood preservatives is one of the major foci of contemporary wood science. Pyrolysis distillates are potential intermediates to extract large volumes of bioactive chemicals. The aim of this study was to characterize pyrolysis distillates from spruce and birch bark and hemp and test different fractions as potential antifungals to prevent wood decay. In all the fungi tested, distillates of spruce caused over 40% inhibition at 0.1% concentration; significant inhibition could be observed when the concentration of the distillates in growth media was 1%. The results indicate that inhibition was caused by the synergetic action of different chemicals in the pyrolysis distillates. When the individual components were considered, propionic acid exhibited a very high inhibitory effect against the wood decay fungi.

The high inhibition of the pyrolysis distillates at 1% and lower concentrations demonstrate that pyrolysis liquids could be a source for formulations of sustainable wood preservatives.

Keywords antifungals; biorefining; decay fungi; thermic liquids; wood decay; propionic acid

1. Introduction

Wood is used for a large range of purposes varying from its traditional use as a structural material to a source of green chemicals via biorefining. Its tendency for degradation due to different abiotic and biotic factors limits its durability when used outdoors. The decay resistance of wood can be improved by chemical treatments that slow down deterioration of its mechanical properties and appearance. The most common chemical preservatives, in either industrial use or emerging from research as potential green substitutes, can be organized into three groups – copper-based preservatives, organic fungicides and insecticides in microemulsions, and the water- and solvent-based preservatives (Coggins, 2008).

Many wood preservatives have been substituted due to performance issues or their adverse effects on human health, thus leading to the current highly regulated operational environment regulations. The trend for increasing legislations on chemicals and sustainability requirements are forecasted to lead to further limitations, as already seen in the case of coal tar creosotes (Hiemstra et al., 2007), chromated-copper-arsenate (CCA) (Mohajerani et al., 2018), and boron-based compounds (Hu et al., 2017).

Use of wooden materials contributes to positive environmental effects via substitution of other, less sustainable materials, but impregnation with traditional wood preservatives increases product toxicity and makes wood lose some of its competitiveness (Werner and Richter 2007). When the life cycle of an untreated wood product ends, it can be revalorised for new uses and finally regarded as an energy source. However, disposal of preserved wood is

48 similar to any hazardous waste, due to the presence of the impregnated toxic chemicals, such
49 as CCA (Augustsson et al., 2017). Nevertheless, in many countries, not all the impregnated
50 wood is submitted to the waste management system as some of it is unofficially reused or
51 burned with nontoxic waste (e.g. Augustsson et al., 2017), causing that the used preservatives
52 are released to the environment. The new generation metal-free wood preservatives have
53 been identified as a possible solution for reducing these negative environmental impacts
54 (Werner and Richter, 2007). Thus, green chemicals that protect wood and prolong its service
55 life in low concentrations would reduce the negative impact of wood treatment and facilitate
56 its recycling and energy use.

57 The development of green chemicals is being undertaken to find cleaner and more sustainable
58 substitutes for the traditional chemicals that are today, or expected to become, forbidden in
59 many commercial applications. Several plant origin chemicals have been found to be
60 successful against wood-decaying fungi, such as essential oils (Xie et al., 2017), tannins (Anttila
61 et al., 2013; Tondi et al., 2015) and extracts of Cameroonian woods (Saha Tchinda et al., 2018)
62 and *Eucalyptus* spp. (González et al., 2017). Furthermore, valorisation of bioactive chemicals
63 from industrial by-products gains importance in this area, as spent coffee extracts (Barbero-
64 López et al., 2018), because biomass is considered the cheapest and most abundant resource
65 that can be found in large volumes (Temiz, 2010). In a recent study, Hokkanen et al. (2014)
66 found over 150 chemicals in the barks of different tree species, which implies refining
67 opportunities in fungicide or pharmaceutical markets. Industrial scale applications for bark
68 derivatives remain few, including painkiller preparation (Vane, 2000; Vane and Botting, 2003)
69 and applications against plant pathogens (Mulholland et al., 2017). As heartwood is rich in
70 phenolic extractives and tannins and it is naturally durable (Scheffer and Cowling, 1966; Taylor
71 et al., 2002), chemicals from heartwood have been extracted to develop natural wood
72 preservatives (Lu et al., 2016). Even though similar chemicals can be found in the bark, extracts

or distillates derived from it have received less attention until quite recently, either as fixing agents or as active antifungals (Tascioglu et al., 2013; González-Laredo et al., 2015).

Thermal processes, such as pyrolysis, are used to degrade solid biomass to liquid pyrolysis distillates, while also producing synthesis gases and solid charcoal (Mourant et al., 2007). These liquid distillates have a number of identified chemical components that are used in consumer products. Along with hot water or other solvent extraction and hydrothermal liquefaction, pyrolysis can be considered as an effective way to extract and convert woody biomass to liquid chemicals that possibly have antifungal properties. Mourant et al. (2005) tested the inhibition caused by pyrolysis distillates produced at 450 °C against four types of wood-decaying fungi using a mix of softwoods. The distillates exhibited versatile response activities depending on the type of fungi. Mohan et al. (2008) tested distillates produced at 400 °C and 450 °C from pinewood, pine bark, oak wood, and oak bark against two wood decay fungi and confirmed the good capacity of the distillates in hindering fungal growth. Lourençon et al. (2016) recently found that impregnating wood with pyrolysis distillates produced at 500 °C from rejected Eucalypt wood fines from a pulp line reduced the water absorption of pinewood and made it more resistant to wood decay.

The present study illustrates the potential of distillate fractions obtained by the slow pyrolysis of Norway spruce bark, silver birch bark, and hemp stem as antifungals against wood-decaying fungi. The effectiveness of the pyrolysis distillates was assessed *in vitro* by growing wood-decaying fungi in contact with diluted pyrolysis distillates, commercial copper-based wood preservatives, and with no inhibitory chemicals. Significant differences between the wood distillates' ability to inhibit fungal growth and minimum inhibition concentrations (MICs) required were observed along with the effect of fungal strain on the inhibition efficiency of the distillates.

2. Materials and methods

98 *2.1. Pyrolysis of bark and hemp*

99 Bark samples of two of the main tree species growing in Finland, Norway spruce (*Picea abies*)
100 and silver birch (*Betula pendula*), and fibre hemp (*Cannabis sativa*), which is grown in Finland,
101 were ground and compacted for processing them in a slow pyrolysis chamber.

102 Slow pyrolysis equipment with an automated operating and condensing temperature control
103 was used with a CO_2 carrier gas flow of 2 L/min for processing. The materials were slowly
104 heated from the room temperature (20 °C) to a maximum operating temperature of 350 °C.
105 Slow pyrolysis was carried out up to the maximum operating temperature in three phases – a
106 drying phase (up to 135 °C), torrefaction phase (up to 275 °C), and pyrolysis phase (up to 350
107 °C). Raw distillates were collected at three nominal condensation temperatures of 130 °C, 70
108 °C, and 5 °C.

109 For each feedstock, distillate fractions were chosen for inhibition testing from torrefaction and
110 pyrolysis phases condensed below 100 °C (Table 1). Distillates 2 (spruce bark) and 4 (birch
111 bark) had two phases each, a water-soluble phase and an insoluble part (oily phase). As the
112 insoluble phase was considered to have a higher concentration of water-insoluble compounds,
113 this phase was taken to represent distillate 2. In the case of distillate 4, a mixture of both
114 phases was considered due to the very low volume of the insoluble phase. The yield of each
115 distillate fraction is provided as liquid fraction obtained from a dry mass of feedstock at the
116 given temperatures.

117 (Table 1)

118 *2.2. Chemical composition of the distillates*

119 The chemical composition of the liquid distillates was analysed using a high-resolution ^1H
120 nuclear magnetic resonance (NMR; Bruker Ascend 600) spectrometer with N_2 filling. The NMR
121 spectra were analysed using TopSpin 3.5 software (Billerica, Massachusetts, USA). Further, the

spectra were phased manually and the baseline correction was included.

Correct identification of peaks was confirmed by other similar samples not discussed here and possible overlapping of signals was taken into account. High resolution of this spectrometer is a relevant advantage. The signal of trisodium phosphate (TSP) was set at 0 ppm and methanol D4 signal was set at 3.30 ppm for the chemical shift scale. The integrated peak areas were converted to concentrations using the signal from TSP, concentration of solvent D4 and number of protons in a specific compound. The detailed procedure is discussed in the forthcoming article (Salami et al. unpublished data).

2.3. Inhibition test

2.3.1. Inhibition test materials and chemicals concentrations

Three species of brown rot fungi, *Coniophora puteana* (strain BAM 112), *Rhodonia (Poria) placenta* (strain BAM 113), and *Gloeophyllum trabeum* (strain BAM 115), were used in this study. Brown rot species were used because they usually decay softwoods, the most commonly used woods for outdoor purposes. The chemicals were tested at concentrations of 0.1%, 0.3%, and 1% (w/w) and were compared to control samples without fungal growth inhibitors, and to a commercial AB-class wood preservative, Celcure C4 (Koppers Inc., Pittsburgh, USA). This industrial reference contained copper(II) carbonate (17%), ethanolamine (<35%), benzalkonium chloride (4.75%), cyproconazol (0.096%), sodium nitrite (<5%), and polyethoxylated tallow amine (<5%).

2.3.2. Fungi breeding method

The three fungal species were grown in 4% malt powder and 2% agar culture media at (22 ± 2) °C and 30% ± 5% relative humidity. Once the mycelium covered the whole surface of the Petri dish, the fungi were stored in a fridge (10 °C). They were taken back to the growing chamber 2 days before using them in the inhibition test.

146 *2.3.3. Preparation of growth media for the inhibition test*

147 Fungal inhibition test was conducted on Petri dishes (Ø 90 mm). The distillates, reference
148 chemicals and later also propionic acid (99%; Merck KGaA, Darmstadt, Germany) were dosed
149 on the fungal growth media. The growth media was prepared by high-intensity mixer by
150 combining the distillates with malt and agar in MilliQ water according to modified version of
151 the method used by Belt (2013). A mix of 4% malt powder, 2% agar, and each distillate in turn
152 at 0.1%, 0.3%, and 1% (w/w) were prepared. Distillate pH was adjusted to 6 by adding 1 M
153 NaOH to allow solidification of the malt-agar media. The growth solution was autoclaved (120
154 °C, 15 min), 20 mL of the mix casted under sterile conditions and the homogeneity of casted
155 plates was monitored visually. Following the same procedure, a malt-agar mix with the
156 industrial copper-based preservative was used as a reference (referred from now onwards as
157 copper reference); growth media with only malt and agar was used as the control referred
158 from now onwards as control.

159 *2.3.4. Fungal inoculation and monitoring*

160 Using a plug, one spherical piece of fungus (ca. 0.238 cm^2, 5.5 mm in diameter) was inoculated
161 on Petri dishes in sterile conditions. The dishes were sealed with parafilm and incubated in a
162 climate chamber with no light at (22 ± 2) °C and 30% ± 5% relative humidity. The area of fungal
163 hyphae growth, i.e. growth rate, after inoculation was measured daily until the grown
164 mycelium of the control samples reached the edge of the Petri dish (21 days for *C. puteana*, 15
165 for *G. trabeum* and 13 for *R. placenta)*. Pictures of the petri dishes were taken with the set up
166 detailed in Ancin-Murguzur et al. (2018). Fungal growth inhibition was measured by modifying
167 the formula proposed by Chang et al. (1999).

168 $\text{Inhibition (\%)} = (1 - (AT - IA)/(AC - IA))*100$

169 Here, AT is the area of the experimental plate or copper reference, AC is the area of the
170 control plate, and IA is the surface area (mm^2) of the inoculated plug.

2.3.5. Data analysis.

For each chemical test sample, copper reference, and control, 10 replicate dishes were prepared and fungal inhibition was calculated based on their mean values. Statistical analysis was carried out using IBM SPSS Statistics 23. Tukey´s range test was used as post-hoc for ANOVA to compare the inhibition of different distillates and copper with respect to the growth in the control specimen. The logarithmic correlation between the different constituents present in the distillates and the inhibition-% caused by the highest distillate concentration (1%) was modelled with SigmaPlot 13.0. No data modifications were needed to perform the statistical analyses: samples were considered independent (i.e. each inhibition rate is independent from the others), normality was considered a non-influential parameter (see Schmidler et al., 2016), and homoscedasticity was not tested for the dataset, as every antifungal agent had the same sample size (n=10) (see Coombs et al., 1996).

3. Results

3.1. Chemical composition of the distillates

The yield of distillates varied significantly depending on the used feedstock and process temperature used. The hemp side-stream had significantly higher yield than wood bark. The chemical composition of the distillates varied according to the concentration of the components. The contents of propionic acid, acetic acid, methanol, formic acid, hydroxymethylfurfural, and furfural in the tested distillates are detailed in Table 2. Water was found to be present in all the distillates. The amount of water in the spruce distillate could not be determined because the peak corresponding to water was suppressed during ^{1}H NMR measurements to enhance the clarity of the signals corresponding to other chemical compounds.

Ethanol was detected in all the distillates except in those extracted from hemp, which had the highest concentration of methanol. Apart from water, which was the major constituent in distillates, acetic acid was the dominant chemical in all the distillates except in distillate 1. Both specimens from birch exhibited high concentrations of acetic acid. The overall chemical content in distillate 1 was low compared to other distillates. The concentration of furfural was low in all the distillates. Propionic acid was found to be present in higher quantities in the distillates isolated during the later stages of the pyrolysis of the same raw material.

(Table 2)

3.2. Inhibition test

All the tested distillates effectively inhibited the growth of *C. puteana* during the Petri dish test (Table 3). At a concentration of 0.1%, the distillates did not cause as much inhibition as the copper reference, which led to almost 100% inhibition. In distillates 1, 4, and 5, the inhibition of fungal growth was not significant (see tables A.1-A.3 in appendices for statistical comparison of the treatments). Distillates 2 and 3 caused a very significant growth inhibition ($P \leq 0.01$). Nevertheless, none of them exhibited 50% inhibition at the lowest dose. Distillates 4 and 5 slightly promoted the growth of *C. puteana* instead of inhibiting it, but the difference with respect to the reference was not significant.

At a concentration of 0.3%, all the distillates caused a very significant decline in the growth of *C. puteana,* with distillates 1 and 5 being the least effective. Distillate 4 exhibited 100% inhibition, similar to the copper reference. Distillates 3 and 6 led to an inhibition of ~95%, while distillate 2 exhibited a mean growth inhibition of 70%.

At a concentration of 1%, the growth of *C. puteana* was completely restricted by all the distillates except distillate no. 1.

(Table 3)

219 The inhibitory effect of the studied distillates on *G. trabeum* (Table 3; Table A.2) was, in
220 general, lower than that on *C. puteana*. At a concentration of 0.1%, distillates 1 and 3 did not
221 show significant growth inhibition, while distillates 5 and 6 caused moderate inhibitions of 10%
222 and 12%, respectively; these results differed significantly with respect to the control. Distillate
223 4 caused a very significant inhibition of 29% and distillate 2 exhibited the highest inhibition of
224 65%. The copper reference exhibited an inhibition of 100% at all the tested concentrations
225 from 0.1% to 1% and differed very significantly from the control sample and its distillate
226 counterparts of the same concentration.

227 When the distillate concentration was 0.3%, distillates 1 and 3 did not cause significant
228 inhibition in the growth of *G. trabeum*. Distillate 4 caused a very significant inhibition of 25%,
229 while distillate 5 caused an inhibition of 35%. The inhibition caused by distillate 6 was 45% and
230 the most potent distillate was no. 2 with a mean inhibition value of almost 86%, which differs
231 very significantly from the control and other treatments.

232 At a concentration of 1%, distillate 1 did not differ significantly from the control. All the other
233 distillates exerted a very significant effect against the growth of *G. trabeum* (see fig 1 for a
234 practical example); distillates 2, 4 and 6 showed the best performance as growth inhibitors
235 with 100% inhibition.

236 (Fig 1)

237 The tested distillates also inhibited the growth of *R. placenta* fungus (Table 3; Table A.3)
238 although the inhibition effect at low concentrations was not as good as in the case of *C.*
239 *puteana* or *G. trabeum*. At a concentration of 0.1%, only distillate 2 caused a significant
240 inhibition in this species, with a mean value of 54%. On the other hand, the copper reference
241 led to 100% inhibition at all the studied concentrations.

242 At a concentration of 0.3%, only distillates 2 and 4 and the copper reference differed
243 significantly from the control. The mean inhibition caused by distillate 4 was 14%, which

differed significantly from all the other treatments. Distillate 2 and the copper reference showed 100% inhibition.

At 1% concentration, all the distillates, except distillate 1, showed a clear inhibitory effect. Distillates 2, 4, and 6 and the copper reference caused total inhibition of *R. placenta*, differing very significantly from the other distillates and control. Surprisingly, distillate 1 failed to have any fungicidal effect at this concentration.

The minimum inhibition concentration (MIC) required to completely inhibit fungal growth was estimated for each distillate. The copper reference reached its MIC when applied at a concentration slightly over 0.1%. The best performing distillate was no. 2, which had a MIC of ca. 0.5%. Distillate 4 exhibited a MIC slightly less than 1%, while those of distillates 5 and 6 were around 1.5% and 1.1%, respectively. In the case of distillates 1 and 3, the MIC values would be very high, which is not feasible in practical applications.

3.3. Contribution of independent distillate constitutes to fungal growth inhibition

There was a weak correlation between the constituent concentration and inhibition activity in the case of *C. puteana* (table 4), but a significant correlation could be observed between fungal growth inhibition and distillate constituent concentration of with respect to propionic acid (R^2 = 0.95 for *G. trabeum* and 0.86 for *R. placenta*). Correlations observed for the other distillate constituents were low.

(Table 4)

3.4. Antifungal efficiency of propionic acid

The propionic acid caused a high inhibition in the three wood-decaying fungi (Table 3). At a propionic acid concentration of 0.1%, the growth of the three fungi species was completely suppressed. As the copper reference caused a total growth inhibition, no significant differences were found between propionic acid and copper reference for these fungi. In the

case of *C. puteana,* propionic acid caused a significantly higher inhibition than the copper reference, with mean inhibition of 100% and 99%, respectively. The visual assessment found that *C. puteana* started to grow in the media with the copper reference the last days of the experiment, but no fungal growth was seen in the plates amended with propionic acid. The MIC value of the propionic acid to completely inhibit the fungal growth of all the studied fungi was estimated to be 0.1% or below, the lowest of all the studied chemicals.

During experimentation, the formation of halos around the mycelia was observed a few days after the placement of a fungal plug on the Petri dishes (Fig 2). These halos were never found in media treated with distillate 1, but they were present around *C. puteana* plugs at higher concentrations (0.3% and 1%) of distillate 3, 4, 5, and 6 and at all concentrations of distillate 2; a similar phenomenon was observed in the case of the copper reference at concentrations of in 0.1% and 0.3%. Halos formed around *C. puteana* plugs were frequently weak, often hard to see by naked eye (see Fig 2B and 2C for weak halo examples). *Gloeophyllum trabeum* exhibited a halo only with distillate 5 at a concentration of 0.3%. *Rhodonia placenta* exhibited no halos with distillates 1 and 3. Halos were observed around the fungal plug in the media treated with distillate 4 at 1% concentration and in the media treated with distillate 5. In the media containing distillates 2 and 6, halos were found around the fungal plugs at all concentrations. A dark-colored halo was also observed in the Petri dish containing the copper reference at a concentration of 0.1%. Halos formed around the *R. placenta* plug were mostly strong and easy to see by naked eye (see Fig 2A for a strong halo example).

(Fig 2)

4. Discussion

Depending on the processing parameters of the slow pyrolysis, about 30–50% of the dry mass is converted to biochar and the rest yields to liquids or non-condensed gases. Conventionally

these liquid and gas fractions are used in energy production and considered secondary to charcoal manufacture. Here we used the distillate fractions at low concentrations to explore alternate utilization pathways with higher benefit and longer life cycle, and consequent carbon storage. The yields of these liquids obtained were relatively high, i.e. at the same level as the amount of extractives in the wood and hemp. The slow pyrolysis has overlapping temperature regime with that of fast pyrolysis, more commonly used to produce thermic liquids from biomass, but still the liquid side-streams obtained have scarce end-uses as a source of chemicals.

The obtained results suggest that the studied distillates suppress the growth of wood-decaying fungi or delay wood decay, as described previously by several researchers (e.g. Mourant et al., 2005; Mohan et al., 2008; Lourençon et al., 2016). The antifungal effect of the distillates varied significantly depending on composition of the distillates, which in turn depends on the raw material and processing conditions. Further, the effect of the distillates was also markedly different towards different fungal species, which is to be expected due to different metabolic rates or the enzymes the fungi release. For example, distillate 3 exhibited excellent performance against *C. puteana*, but poor activity against *G. trabeum* and *R. placenta*.

Unlike other studies in which experiments were conducted at high distillate concentrations (Kim et al., 2012; Temiz et al., 2013), the distillate concentrations used in this study were intentionally kept low to explore the potential of low-concentration solutions in preventing or inhibiting wood decay. Distillate 2 from the second phase of the pyrolysis of spruce showed good performance against various types of decay-causing fungi. However, when the concentration was increased, other distillates were more effective. Pyrolysis phase distillates typically show a higher activity than torrefaction-produced distillates. The torrefaction-produced distillate from hemp (distillate no. 5) was, in contrary, clearly more effective than the corresponding distillates from spruce and birch bark (distillates 1 and 3, respectively).

318 Furthermore, if the distillate phases are separated and tested individually, the oily phase tends
319 to contain significantly higher concentrations of oil- soluble active compounds.

320 Several previous studies suggested a relationship between fungal growth inhibition and the
321 content of phenolics in the distillates (Mourant et al., 2005; Baimark and Niamsa, 2009; Temiz
322 et al., 2010; Kim et al., 2012; Theapparat et al., 2014). Several phenolic compounds play an
323 important role in the natural decay resistance of wood (Harju et al., 2003; Rättö et al., 2004).
324 Oramahi and Yoshimura (2013) suggested that the total acid content of the distillates
325 increased at higher pyrolysis temperatures and this influenced their antifungal nature.
326 Furthermore, it was observed that chemicals extracted from the same feedstock at higher
327 temperatures had a higher impact on fungal growth. This can be attributed to the large
328 number of methanol, formaldehyde, and complex tar compounds in pyrolysis distillates
329 obtained at higher temperatures compared to the distillates obtained at temperatures below
330 200 °C, where they contain higher amounts of organic acids and water.

331 The comparison for the composition of starting biomass and the composition of pyrolysis
332 distillates on molecular level is considered out of the scope of this study. Simple distillate
333 constituents, such as acetic acid, have also been found to delay wood decay (Bahmani et al.,
334 2016). Acetic anhydride and furfural alcohol used for creating acetylated and furfuralated
335 wood of high decay resistance also embody the benefits of acetic acid, furfural, and HMF in
336 distillates (Mantanis, 2017). The results reported by Kim et al. (2012) suggest that together
337 with phenolics, organic compounds protect wood from decay by penetrating and
338 agglomerating inside the wood material. Additionally, Fagernäs et al. (2012) highlighted that
339 the presence of acetic acid and furfural in distillates can make these liquids potent natural
340 pesticides. Based on our results and earlier findings, it is reasonable to suggest that the
341 synergetic action of acidic and phenolic chemicals is behind the best antifungal performance of
342 distillates.

343 At the highest distillate concentration, the strongest correlation was noted between the
344 concentration of propionic acid in the distillates and growth inhibition of fungi. After a test
345 with propionic acid dose in growth media, it was noted to suppress the growth of the studied
346 fungi already at 0.1%. Propionic acid has been tested against moulds by Kiesel as early as in
347 1913. A study by Bahmani et al. (2016) showed recently that propionic acid acts against several
348 molds and decay by *Pleurotus ostreatus* and *C. puteana* in date palm (*Phoenix dactylifera*) and
349 oil palm (*Elaeis guineensis*). Our analysis agree with these results and highlights that the fungal
350 inhibition caused by propionic acid alone is statistically the same as the inhibition caused by
351 the copper reference, as they do not differ significantly. Nevertheless, the visual analysis of the
352 samples showed that *C. puteana* inoculums were starting to grow the last days of experiment
353 in the media amended with the 0.1% copper reference, while they did not start growing in the
354 media with 0.1% propionic acid. This indicates a great potential for the applications of
355 propionic acid and warrant further studies.

356 The presence of halos around *C. puteana* and *R. placenta* may indicate that these fungi release
357 oxidising chemicals, metabolites, or other compounds to detoxify the growth media from
358 constituents toxic to them (Lee et al., 1992; Rabinovich et al., 2004; Morel et al., 2015).
359 Previous reports suggest that fungi grown in a Petri dish release compounds to transform the
360 chemicals present in the media. For example, *Alternaria alternata* and *Botrytis cinerea* detoxify
361 any copper present in the media by releasing siderophores to create colorful halos (Kovačec et
362 al., 2017); such observations corroborate our findings.

363 Propionic acid independently performed considerably better than the pyrolysis distillates as a
364 fungal inhibitor. The MIC values for complete inhibition of all the tested fungi were 0.1% for
365 propionic acid, and between 0.5% and 1% for the best performing distillate fractions. However,
366 the use of distillates directly would provide a much cheaper opportunity for wood preservative

367 formulations than the propionic acid isolation and purification, although other propionic acid
368 sources can also be considered.

369 The societal demand regarding the use of non-toxic and sustainable resources drives the
370 development of sustainable alternatives in materials and energy production (Chen et al.,
371 2017). We found that some of the tested pyrolysis distillates have high inhibitory effects
372 already at 0.1% concentration, which can be considered low. The use of virtually any chemical
373 in wood preservation or modification increases the environmental impact of obtained wood
374 products for that of native wood (Werner and Richter, 2007) and the magnitude of that impact
375 is defined by the type and volume of chemical used. The bio-based wood preservatives are
376 believed to have lower negative environmental impacts than the ones used today (Ding et al.,
377 2017). The pyrolysis distillates could fill some of this need due to their high antifungal activity,
378 becoming a promising source of greener wood preservatives or cleaner, renewable origin for
379 antifungal chemicals.

380 The agar plate testing method used in this study measures, primarily, the acute toxicity of the
381 distillate compounds, i.e. their interference with the basic metabolism of the fungus. However,
382 the toxicity of an organic compound needs not be at the same level as that of the reference
383 compound, in this case, copper. For a feasible low- or non-biocidal wood preservative, direct
384 metabolic toxicity is not the only way in which fungal degradation can be prevented. The total
385 performance of an environmentally benign preservative could be a synergetic effect of water
386 repellence, antioxidant activity, interaction with metal ions, and fungicidal properties (Binbuga
387 et al., 2008). Therefore, the performance of a new preservative formulation should be verified
388 by decay tests using impregnated wood materials. Furthermore, decay tests with wood
389 specimens are necessary to prove that the fungicidal effects of the compounds materialize
390 even when they are integrated in wooden substrates (Loman, 1970).

391

5. Conclusions

Synergetic effect of organic acids and phenolics found in slow pyrolysis distillates exhibit antifungal activity against wood-decaying fungi even at low concentrations. The pyrolysis process stage at which a distillate is extracted significantly affects its composition and effectiveness in inhibiting fungal growth. For individual distillate components, the propionic acid was the most effective avoiding the growth of fungi already at 0.1%. Pyrolysis distillate components could be an alternative resource for wood preservative formulations. Further studies are needed to understand and possibly mitigate fungal detoxification strategies against these chemicals and their performance as preservatives with wood specimens.

Supplementary data: E-supplementary data of this work containing the statistical comparison of distillate-induced growth inhibition of fungi can be found in online version of the paper.

Declarations of interest: none

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Tables

Table 1: Different distillates obtained by pyrolysis and their source feedstock

Distillate	**Feedstock**	**OP-Temp (°C)**	**C-Temp (°C)**	**Holding time (h)**	**Mass yield (%)**
Distillate 1	Spruce bark	275	<100	17	5.7
Distillate 2	Spruce bark	350	<100	6	2.9
Distillate 3	Birch bark	275	<100	13	3.1
Distillate 4	Birch bark	350	<100	11	2.8
Distillate 5	Hemp	275	<100	17	5.6
Distillate 6	Hemp	350	<100	5	11.5

*Note: **OP-Temp** = maximum operating temperature and **C-Temp** = nominal condensation temperature.

Table 2: Chemical constituents identified in different distillates derived from tree bark and fibre hemp and their molar concentration (M)

#	Source	Water (M)	Propionic acid (M)	Ethanol (M)	Acetic acid (M)	Methanol (M)	Formic acid (M)	HMF* (M)	Furfural (M)
1	Spruce	-	0.004	0.032	0.084	0.310	0.007	0.005	0.038
2	Spruce	-	0.137	0.030	1.620	0.118	0.022	0.004	0.007
3	Birch	59.9	0.040	0.010	4.930	0.164	0.034	0.050	0.027
4	Birch	54.6	0.180	0.022	4.090	0.390	0.009	0.003	0.020
5	Hemp	29.1	0.170	trace	1.060	1.220	0.030	0.000	0.004
6	Hemp	39.1	0.250	trace	3.780	0.540	0.075	0.024	0.010

** Hydroxymethylfurfural*

Table 3: Inhibition (%) caused by the distillates and copper reference at 0.1%, 0.3% and 1% concentration compared to the growth in control plates. Results are presented as mean inhibition ± SE. Different letters indicate significant differences caused by distillates within each fungus species. The inhibitions over 95% are highlighted in bold as they were considered as excellent performing by the authors

N = 10	Concentration	*C. puteana*	*G. trabeum*	*R. placenta*
Distillate 1	0.1%	9.2 ± 4.5^{ab}	7.7 ± 3.5^{a}	0.0 ± 3.0^{a}
	0.3%	27.7 ± 5.4^{cd}	4.7 ± 1.7^{a}	-0.2 ± 4.5^{a}
	1.0%	26.3 ± 5.4^{cd}	5.1 ± 1.6^{a}	-2.2 ± 2.8^{a}
Distillate 2	0.1%	46.6 ± 2.8^{e}	65.2 ± 1.6^{e}	53.7 ± 2.4^{d}
	0.3%	70.2 ± 4.7^{f}	85.9 ± 1.1^{fg}	**100.0 ± 0.0^{f}**
	1.0%	**100.0 ± 0.0^{g}**	**99.6 ± 0.1^{g}**	**100.0 ± 0.0^{f}**
Distillate 3	0.1%	32.5 ± 6.5^{de}	3.8 ± 1.9^{a}	-2.8 ± 2.6^{a}
	0.3%	**97.7 ± 2.3^{g}**	7.0 ± 3.2^{a}	0.8 ± 4.4^{a}
	1.0%	**100.0 ± 0.0^{g}**	65.5 ± 1.5^{e}	35.4 ± 2.6^{c}
Distillate 4	0.1%	-7.5 ± 1.7^{a}	29.9 ± 5.0^{cd}	5.0 ± 4.7^{ab}

	0.3%	**100.0 ± 0.0^{g}**	25.1 ± 3.9^{bc}	14.5 ± 2.8^{b}
	1.0%	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{f}**
Distillate 5	0.1%	-5.3 ± 4.1^{a}	10.5 ± 3.7^{ab}	-3.5 ± 2.3^{a}
	0.3%	23.9 ± 7.2^{bcd}	34.5 ± 9.5^{cd}	-1.0 ± 3.5^{a}
	1.0%	**100.0 ± 0.0^{g}**	82.5 ± 1.1^{f}	68.9 ± 1.5^{e}
Distillate 6	0.1%	11.9 ± 3.6^{bc}	12.4 ± 1.7^{ab}	-3.9 ± 2.2^{a}
	0.3%	**98.2 ± 1.0^{g}**	45.2 ± 4.0^{d}	-2.7 ± 2.5^{a}
	1.0%	**100.0 ± 0.0^{g}**	**96.8 ± 0.4^{fg}**	**100.0 ± 0.0^{f}**
Propionic acid	0.1%	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{f}**
Copper	0.1%	**99.3 ± 0.2^{g}**	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{f}**
	0.3%	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{f}**
	1.0%	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{g}**	**100.0 ± 0.0^{f}**

Table 4: Logarithmic correlation found between the chemical at highest concentration of pyrolysis distillates

Constituent	*C. puteana* R^2	*G. trabeum* R^2	*R. placenta* R^2
Propionic acid	0.80	0.95	0.86
Ethanol	<0.25	<0.25	<0.25
Acetic acid	0.80	0.69	0.48
Methanol	<0.25	<0.25	<0.25
Formic acid	0.36	<0.25	<0.25
HMF*	<0.25	<0.25	<0.25
Furfural	0.31	0.36	0.33

** Hydroxymethylfurfural*

Figures

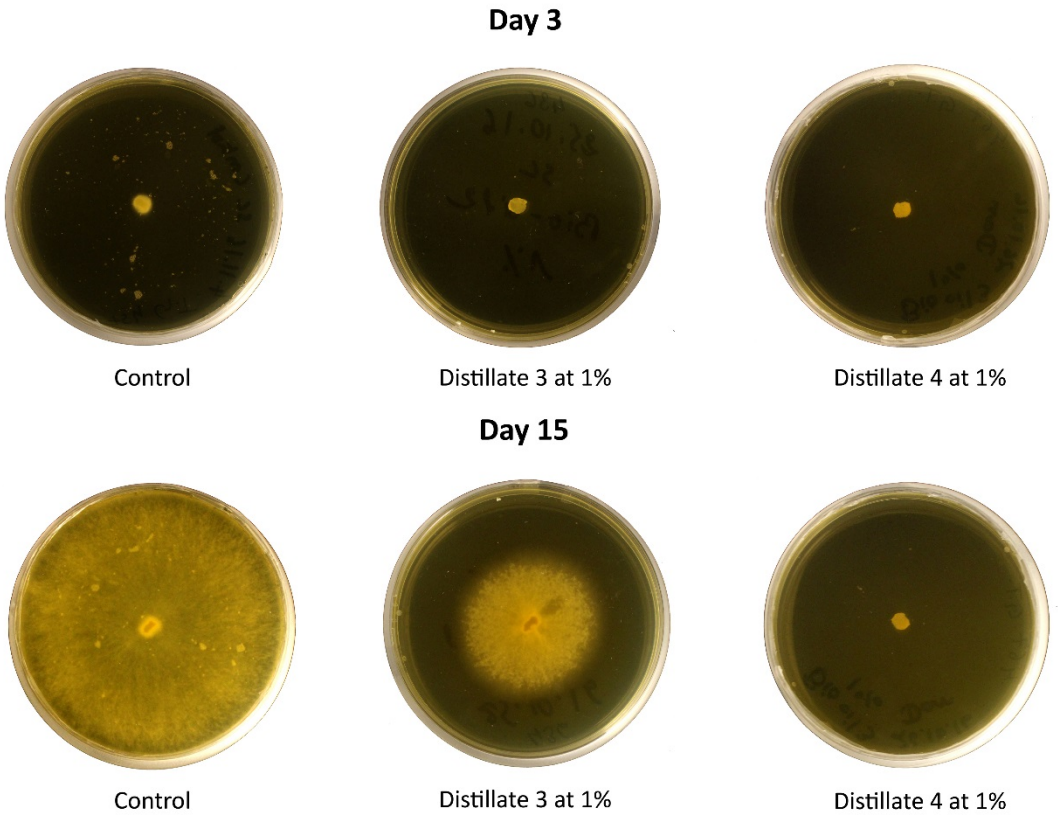

Fig 1: Growth of *G. trabeum* in petri dish with only malt agar, and distillates 3 and 4 at 1%. Above, the growth of the fungus 3 days after inoculation. Below, the same petri dish 15 days after inoculation. For measuring the inhibition, the growth of the fungus in the petri dish were compared to the growth of the same fungus in the control petri dish.

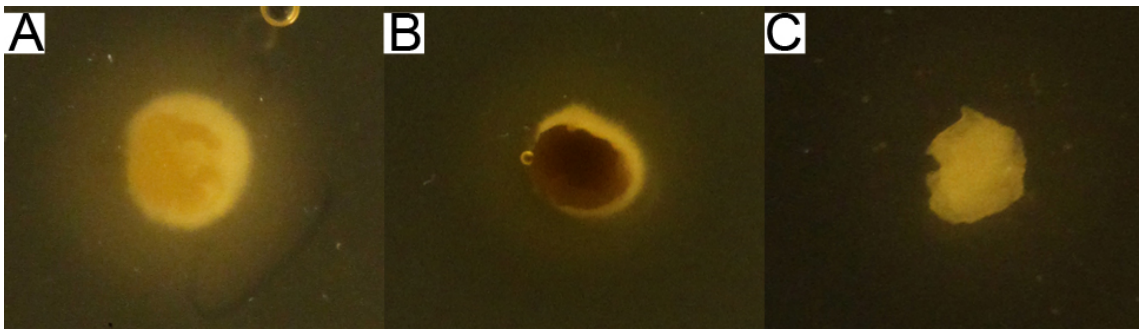

Fig 2: (A) Strong halo around an initial *C. puteana* plug growing in a media amended with distillate 5 (1%) after 5 days; (B) weak halo around a *C. puteana* plug growing in a media

amended with distillate 2 (0.1%) after 3 days; (C) very weak halo around a *R. placenta* plug growing in a media amended with distillate 5 (0.1%) plug after 3 days. The fungal inoculum in the center of the images is 5.5 mm in diameter.