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SHORT COMMUNICATION

Antifungal Activity of Several Vegetable Origin Household Waste Extracts Against Wood-Decaying Fungi In Vitro

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Abstract

Purpose The aim of this paper is to identify if vegetable origin household wastes (VHWs) can be used in wood preservative formulations.

Method An antifungal activity test of the VHW hot water extracts against wood-decaying fungi in vitro was performed.

Results Out of the 17 studied VHW extracts 14 showed some antifungal activity against at least one fungi. Extracts from banana, tangerine and watermelon peels and discarded onion peels inhibited fungal growth the most.

Conclusions The results demonstrate that VHWs are a source of antifungal chemicals, which can be considered as an alternative or additive to present wood preservatives formulations.

Graphic Abstract

Keywords Food solid waste · Domestic waste · Antifungal · Wood degradation · Wood preservation · Biorefining

Statement of Novelty

Forest industry is searching for new wood preservatives, because actual wood preserving chemicals are toxic to the environment and humans. At the same time, the society needs to find new uses to the organic waste generated by private users. The present study aimed to extract the natural chemicals found in these wastes, and test them as antifungals against the fungi responsible of decaying wood. This study found antifungal activity in the extracts of many of the wastes tested, proving that the wood industry could benefit from using organic waste in wood preservative formulations, what would bring new green solution to the forest industry.

Introduction

The quest for new wood preservatives is driven by the increasing tolerance of fungi to copper, the society´s environmental awareness and more strict regulations in chemical legislation in traditional wood preservatives. As some bio-based chemicals are known to be effective wood-decaying fungi inhibitors, such as tannins [1] and wood pyrolysis distillates [2], they have gained attention as substitutes of the traditional wood preservatives.

The increasing concern about the amounts of organic household waste generated all around the world is making research focus in revalorization and recovery of

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food residues. Many vegetable origin household wastes (VHWs), as fruit peels or vegetable parts which are not usually eaten, and are often richer than the edible tissues in different constituents as phenolics, meaning that many bioactive chemicals could be extracted from them [3]. However, very few researchers have studied how wood-decaying fungi are affected by the extracts of some VHW, as spent coffee grounds [4].

The aim of this paper is to test if VHWs can be a source of chemicals for wood preservative formulations. The antifungal activity of the hot water extracts of the VHWs against several wood-decaying fungi was tested in vitro. The results bring new, cheap and sustainable possibilities for extracting antifungal constituents to be used in green wood preservatives. The antifungal constituents could offer an alternative to wood industry to some of the most harmful constituents of present preservatives, what would reduce their negative impact to the environment. Additionally, it would find a use to different wastes that are nowadays underused.

Materials and Methods

Plant Origin Household Wastes and Their Extraction

The VHWs tested in this experiment, coming from food purchased in food stores from Joensuu (Finland), were as follows: Potato, avocado, kiwi, orange, tangerine, banana, honeymelon, triploid watermelon, butternut squash and pumpkin peels; pistachio shell (endocarp) and coconut shell (exocarp); avocado seed; artichoke outer petals; discarded onion peels (scaly leaves and base of scape); spent coffee grounds (100% *C. arabica*) from standard coffee brewing and cold coffee brewing (brewed for 40 h, at 3 ± 1 °C).

All the VHWs except spent coffee were collected by the author of the paper after separating them from the edible parts. These VHWs were oven dried at 50 °C until constant mass was reached, and then crushed. Spent coffee from standard and cold coffee were put into an oven at 50 °C 12 h after being used to brew coffee, and were left in the oven until constant mass was reached. Extractions were done by boiling 15 g of each dried crushed VHW in 300 mL of Milli-Q water (Merck KGaA, Darmstadt, Germany) for 15 min. After the extraction, the solids were removed using a fine sieve and the extracts were levelled up to 300 mL with Milli-Q water as some of the water evaporated while boiling.

Antifungal Test

Antifungal Test Material and Chemical Preparation

Two brown-rot fungi, *R. (Poria) placenta* (strain BAM 113) and *G. trabeum* (strain BAM 115) and a white-rot fungus, *T. versicolor* (strain BAM 116), purchased from the Federal Institute for Materials Research and Testing (BAM, Berlin, Germany), were used for this test.

The growth media for the antifungal test was prepared by mixing 4% malt and 2% agar in each of the VHW extract. Each growth media was then autoclaved (120 °C, 15 min), 15 mL of it casted in petri dish (Ø 90 mm) and cooled down under sterile conditions. A growth media with 4% malt, 2% agar and Milli-Q water was prepared as a control, and a growth media with 4% malt, 2% agar and 1.6% copper-based AB-class preservative (Celcure C4, Koppers Inc., Pittsburgh, USA) was prepared as a commercial reference. The constituents of the commercial copper-based wood preservative were copper(II) carbonate (17%), ethanolamine (< 35%), benzalkonium chloride (4.75%), cyproconazole (0.096%), sodium nitrite (< 5%), and polyethoxylated tallow amine (< 5%).

Fungal Inoculation and Growth Monitoring

The fungal inoculation was done using a plug of 0.28 cm^2 in sterile conditions. The plug was used to put a spherical piece of the previously cultivated fungal strains into the center of the petri dish with the growth media that contained the VHW extract, copper-based preservative and the control media. The petri dishes were then sealed with parafilm and kept in a growing chamber at 22 ± 2 °C and $65\% \pm 5\%$. The area of the fungal colony was measured daily until the fungi growing in the control samples covered the whole petri dish, between 8 and 14 days, depending on the fungal strain and replicates. The set-up of Ancin-Murguzur et al. [5] was used to take pictures of the petri dish every second day. The antifungal activity of the VHW extract was measured following the formula used by Chang et al. [6]:

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

here, AT is the surface covered by the fungal colony in the experimental petri dish (%), AC is the surface covered by the fungal colony in control petri dish (%) and IA is the surface covered by the inoculated plug (%).

Data and Statistical Analysis

Between six and eight replicates were prepared for each VHW extract, copper-based preservative and control sample, and the antifungal activity was measured based on their

mean values. The statistical analysis of the antifungal activity of VHW extract and copper-based preservative compared to controls was performed using IBM SPSS Statistics 23, using Tukey's range test as post-hoc for ANOVA.

Results and Discussion

Most of the VHW extracts inhibited *G. trabeum* mycelial growth by at least 20% from the baseline of pure agar media (Table 1). The results of this study show potential of several of these extracts as antifungals against wood-decaying fungi, agreeing with previous studies that found that some VHW extracts have antifungal activity in wood, such as pomegranate peels [7] and spent coffee ground extracts [4].

Out of all the tested VHW extracts, banana peel extract was the most effective antifungal, as it was the only extract that caused over 50% inhibition in all the studied fungi. This finding agrees with previous studies, which proved that banana peel extracts are successful inhibitors of other kinds of fungi, such as *A. niger* [8]. Saleem and Saeed [9] proved that several micro-organisms, including bacteria, fungi and yeasts were also inhibited by banana peel extracts, what supports the findings of this paper. The high amount of phenolics present in banana peels, which are known antimicrobials, could be responsible of this fungal inhibition [10].

Tangerine peel extract was also a successful inhibitor of wood-decaying fungi as it inhibited *G. trabeum* and *T. versicolor* about 50%, although the growth of *R. placenta* was only inhibited about 10%. Essential oil from tangerine peels are known to inhibit food spoilage related molds [11] and bacteria [12]. Citric acid—present in tangerine peels—is a useful chemical to protect wood from the decay fungus *R. placenta* [13]. Here, this fungal species grew only 10% less than control when exposed to tangerine peel extract, what could be caused by the lower concentration of citric acid in the extract or due to its other constituents, such as carbohydrates, which are present in tangerine peels [14].

Discarded onion peel extracts inhibited *G. trabeum* 69%, *T. versicolor* 45%, and *R. placenta* 22%. The antifungal activity of the phenolic compounds, such as quercetin [15] could be responsible of the growth inhibition of the studied wood-decaying fungi, as they are present in onion peels [16].

Watermelon peel extracts inhibited significantly both brown-rot fungi in this study, but did not inhibit the white-rot fungus *T. versicolor*. Watermelon peel extracts are effective as food preservatives [17] as well as inhibitors of health issue related bacteria and fungi [18]. The results of this work together with previous findings prove that watermelon peel extracts at tested concentration are able to inhibit several fungi, but some others, such as *T. versicolor*, are not sensitive to them.

Table 1 Growth inhibition (%) of *G. trabeum*, *R. placenta* and *T. versicolor* caused by the different VHW extracts and copper-based wood preservative

	Inhibition (%)		
	G. trabeum	*R. placenta*	*T. versicolor*
Potato peels (periderm)	18.0 ± 2.5^{bc}	7.1 ± 2.7^{ab}	-15.8 ± 3.4^{ab}
Orange peels	24.0 ± 2.3^{cd}	0.4 ± 0.8^{a}	26.5 ± 1.9^{f}
Tangerine peels	$\mathbf{56.5 \pm 1.0^{fg}}$	10.1 ± 1.2^{b}	$\mathbf{48.2 \pm 5.8^{g}}$
Honeymelon peels	28.1 ± 2.8^{cde}	1.4 ± 1.0^{a}	-11.5 ± 5.7^{abcd}
Watermelon peels	39.3 ± 5.4^{de}	$\mathbf{59.4 \pm 1.1^{e}}$	0.9 ± 2.2^{cd}
Butternut squash peels	32.8 ± 5.3^{cde}	1.2 ± 1.2^{a}	-9.1 ± 2.7^{abcd}
Pumpkin peels	39.4 ± 4.5^{de}	21.9 ± 1.9^{c}	-0.8 ± 2.5^{bcd}
Banana peels	$\mathbf{61.3 \pm 0.9^{g}}$	$\mathbf{53.5 \pm 0.9^{e}}$	$\mathbf{71.2 \pm 1.7^{h}}$
Avocado peels (exocarp)	30.8 ± 1.0^{cde}	9.3 ± 1.6^{b}	-7.8 ± 3.4^{abcd}
Avocado seed	39.9 ± 2.1^{e}	34.7 ± 1.4^{d}	18.7 ± 2.4^{ef}
Pistachio shell (endocarp)	3.3 ± 3.0^{ab}	6.3 ± 1.4^{ab}	4.9 ± 2.9^{de}
Coconut shell (exocarp)	5.2 ± 2.4^{ab}	0.1 ± 1.0^{a}	-17.8 ± 4.1^{a}
Kiwi peels (exocarp)	18.2 ± 2.5^{bc}	-0.3 ± 2.0^{a}	-1.8 ± 1.7^{abcd}
Artichoke petals	42.1 ± 5.3^{ef}	0.2 ± 0.6^{a}	-11.2 ± 3.4^{abcd}
Discarded onion peels	$\mathbf{69.2 \pm 3.0^{g}}$	22.4 ± 2.1^{c}	44.6 ± 1.7^{g}
Spent cold brew coffee	27.0 ± 1.5^{cde}	26.2 ± 1.6^{c}	25.4 ± 3.4^{f}
Spent normal brew coffee	7.6 ± 3.7^{ab}	6.0 ± 2.4^{ab}	-14.0 ± 3.8^{abc}
Cu-based preservative	$\mathbf{100.0 \pm 0.0^{h}}$	$\mathbf{100.0 \pm 0.0^{f}}$	$\mathbf{100.0 \pm 0.0^{i}}$
Control	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{bcd}

Different letters indicate significant differences caused by the tested chemicals within each fungus species

The inhibition values highlighted in bold were considered the best performing by the author

Avocado seed and spent cold brew coffee extract were effective against the three tested fungi species, as they are constituted by antifungal chemicals, such as phenolics in avocado seed [19] or coffee-derived chemicals—such as caffeine or chlorogenic acid—in spent coffee grounds [4].

The rest of the VHW extracts showed variable performance or did not inhibit significantly the growth of any of the studied fungi. In case of coconut shell extract, it even promoted the growth of *T. versicolor*, although other extraction methods resulted in extracts with wood preserving properties [20]. The reason of the low performance of the extracts in this test is possibly the different metabolisms and tolerances to chemicals of the fungi, the low efficiency of the extraction method for some of the VHW, and the carbohydrates that are present in many of these VHW extracts, such as tangerine peels [14].

The results of this study together with the previous literature indicate that phenolic compounds, together with organic acids, are responsible of the growth inhibition caused to wood-decaying fungi. The antioxidant activity of the VHW

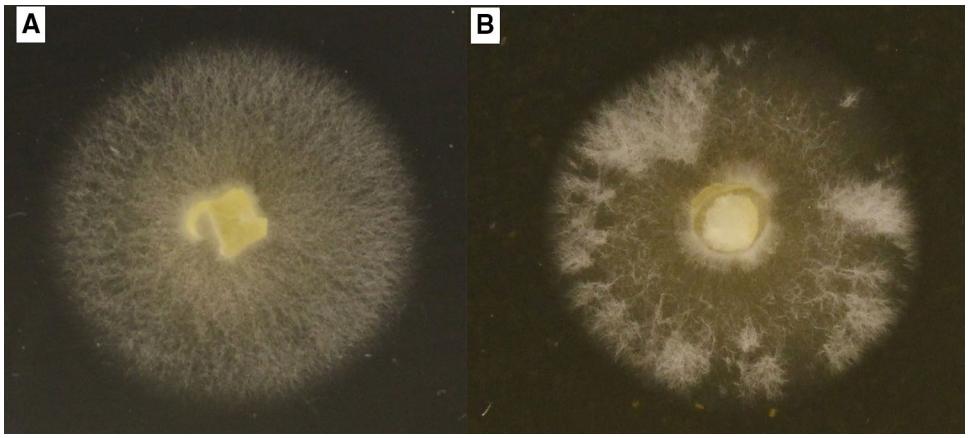

Fig. 1 *Gloeophyllum trabeum* colony growing in **a** control media and **b** media with tangerine peel extract

extracts could be one of the mechanisms behind the fungal inhibition, as previous studies found that antioxidant activity could play a role in inhibiting wood-decaying fungi [21]. In this paper banana peel extracts were the best performing extracts, and are known to be rich in phenolics and highly antioxidant [10], what supports previous findings about the fungal inhibition mechanisms.

During the visual observation of the petri dish, a different colored halo around some of the fungus was detected in some of the petri dish after 3–4 experiment days. The fungus *T. versicolor* generated the halo in the growth media amended with tangerine, banana and avocado peels, avocado seed, artichoke petals, discarded onion peels and spent cold brew coffee. *Rhodonia placenta* generated the halo in pumpkin peel extract, while no halo was seen in any of the media where *G. trabeum* was inoculated. Similar halos have previously been related to chemicals released by the fungi to detoxify the media [4]. The shape of the colony was regular (circular) for all the fungi in all the different growth media except *R. placenta* growing in the media with discarded onion peel extract, which was irregular. The density of the colony in the *G. trabeum* colonies growing in contact with tangerine, pumpkin, banana and orange peel were often variable. During the first 5 to 7 days of the experiment, they presented areas with very low colony thickness, while all the other treatments and controls presented a constant thickness within the colony (see Fig. 1 as a visual example). These irregularities may be related to the stress of the fungi due to the presence of chemicals with antifungal activity.

The antifungal tests in agar plates, such as the one used in this experiment, provide information about how the chemicals tested affect the metabolism of fungi [2]. This test excludes other wood-related factors that may hinder the effects caused by the studied chemicals, such as the wood anatomy or the variability in the distribution of the structural compounds [22]. Before using VHW extracts in wood preservative formulations, further tests are needed, where VHW extract amended wood preservatives are tested for their leaching from wood and performance against wood-decaying fungi.

Conclusions

The studied VHW extracts have antifungal activity against wood-decaying fungi, although their effects vary significantly depending on the studied fungal species and the different extracts. Banana peel extract is the best performing hot water extract tested as it inhibited all the wood-decaying fungi. Onion, tangerine and watermelon peel extracts cause significant inhibition of some fungi, and thus, together with banana peel extract, are the most promising tested extracts for wood preservative formulations, and could provide a cheap and sustainable source of antifungals for the wood industry.

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Data Availability The data will be kept by the author of the paper.

Compliance with Ethical Standards

Conflicts of interest The Author declares no conflict of interest in publishing this manuscript.

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