

UV-absorption Capacity of Selected Crude and Functionalized Lignin for Use in Sunscreens

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ABSTRACT

The most commonly used commercial sunscreen agents are aromatic compounds oxybenzone and octinoxate, as they offer a wide range of protection in the UV spectrum. However, oxybenzone has the highest rate of skin irritation, while octinoxate has poor photostability. Oxybenzone is also a genotoxicant and skeletal endocrine disruptor to corals. Appropriate, less toxic and preferentially biorenewable alternatives should be recommended. Lignin, an abundant byproduct of pulp and biorefinery industries, is expected to be an effective replacement for oxybenzone and octinoxate due to its aromatic structure, which, in synergy with a wide variety of functional groups, produces the absorption of energy in the entire UV region. Additionally, lignin is a biocompatible polyphenolic with a strong radical quenching ability, i.e., anti-oxidizing potential. Therefore, it is a promising replacement for synthetic chemicals in cosmetics, sunscreen, and other applications. The UV absorption analysis of selected crude and functionalized lignin samples was conducted, and the results were compared to those of commercially used UV-absorption agents—oxybenzone and octinoxate. The results showed that the total absorption capacity of the lignin samples is lower by 6.4x and 16.3x compared to oxybenzone and octinoxate, respectively; however, it covers a wide absorption range in the UV spectrum. The lignin samples also showed good

photostability and color stability, in contrast to the observed yellowing of octinoxate after 3 hours of exposure to sunlight. The UV spectra of some of the lignin samples indicate that their UV absorption capacity was enhanced by as much as 13.31% after exposure to sunlight.

Keywords: Absorbance spectra, bio-renewable sunscreen, hot water extraction, lignin, UV absorption capacity

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INTRODUCTION

Lignin is the most abundant natural aromatic polymer and is the second most abundant polymer, next to cellulose. The Department of Energy targets a 30% replacement of fossil fuels with biofuels by 2030, which equates to 0.225 billion tons of lignin (Holladay et al., 2007). As a byproduct of biorefineries, Lignin is currently utilized mostly as feed for boilers for energy production. However, lignin has the potential to be developed into higher-value products, which will help increase the profitability of biorefineries (Dhiman et al., 2018). In these applications, the native structure of lignin is preferred over sulfur-containing and more condensed lignin from industrial operations. Mild processes like hot water extraction (HWE) are recommended to produce lignin with properties that can be altered for desired higher-value applications (Bujanovic et al., 2012).

Lignin possesses amphiphilic properties because of its abundance in hydrophilic groups (hydroxyl groups, carboxyl groups) and hydrophobic structural units (benzene rings, alkyl chains) (Zhang et al., 2019). Lignin is known to have UV-absorbing properties due to its phenolic units and thus has been integrated into different UV protection applications (Lee et al., 2019). Studies showed that lignin has a high antioxidant capacity with no cytotoxic effect over various concentrations (Gil-Chávez et al., 2019; Ugartondo et al., 2008). Lignin has antioxidant capacity due to its wide variety of functional groups (phenolic and aliphatic hydroxyls, carbonyls, carboxyls) as well as its phenylpropanoic structure. They interfere with sequential free radical reactions, reducing the risk of DNA and membrane damage (García et al., 2017; Randhir et al., 2004). Therefore, lignin can be used in sunscreen lotions and other cosmetics as a biocompatible and natural alternative to commercial sunscreen agents.

Although beneficial for vitamin D synthesis and immune development, ultraviolet radiation poses clinical acute and chronic effects on humans, which include erythema (sunburn), pigmentation, suppression of acquired immunity, photocarcinogenesis and photoaging. Thus, sunscreens were developed (Young et al., 2017). However, commercial sunscreen agents such as para-aminobenzoic acid (PABA) cause irritation, contact allergies, and skin staining. On the other hand, cinnamates like octinoxate need frequent reapplication due to their poor photostability. Additionally, benzophenones such as oxybenzone are good UV-absorbing agents; however, they also set the record for the highest rate of causing allergies (Sambandan & Ratner, 2010). The Hawaiian State Legislature recently banned sunscreens containing oxybenzone and/or octinoxate to preserve marine ecosystems. A study by Downs et al. (2015) showed that increasing concentration of oxybenzone increases the rate of coral bleaching and is a genotoxicant and skeletal endocrine disruptor to corals. Downs et al. (2015) concluded that "oxybenzone poses a hazard to coral reef conservation and threatens the resiliency of coral reefs to climate change." Thus, there is a need to develop a natural, non-toxic, and environmentally friendly alternative to this commercial sunscreen agent.

Studies about the feasibility of lignin in UV protection applications have been broadly explored. However, as far as researchers know, lignin's total UV absorption capacity has not yet been widely investigated. In this study, the selected lignin is pure lignin isolated through weak acid hydrolysis from a commercial facility, sugar maple and willow lignins recovered from HWE and functionalized willow lignins. The main objective of this study was to determine the total UV absorption capacity of the selected crude and functionalized lignin and compare it to oxybenzone and octinoxate. Specifically, this study sought to:

1. Determine the absorbance spectra of the lignin samples and the commercial sunscreen agents.
2. Investigate the color- and photostability of the lignin samples and compare them to the commercial sunscreen agents.
3. Determine the absorptivity coefficients of the lignin samples in a chosen solvent, DMSO, and
4. Investigate the potential of the lignin samples as broad-spectrum UV absorption agents.

MATERIALS AND METHODS

Materials/Reagents

Five lignin samples were collected: (1) pure lignin isolated through weak acid hydrolysis from a commercial facility (PL), (2) sugar maple lignin recovered from hot water extract (SMRecL), (3) willow lignin recovered from hot water extract (WRecL), (4) acetylated willow lignin (WAc), and (5) fatty acid esterified willow lignin (WFAE). Supplementary data can be obtained from the publisher to process each lignin sample. Commercial sunscreen agents for UV absorption, oxybenzone and octinoxate, were purchased from Sigma-Aldrich.

Preparation of Solutions

It was washed with a sulfuric acid solution of pH 5 to remove the impurities in the PL sample. A binary solvent system of 9:1 (v/v) acetone/water was used to dissolve all the lignin samples and the oxybenzone. Solutions of 0.4% wt/v were prepared for each set-up. The octinoxate is liquid at room temperature, while the rest are in powdered form. Each experimental set-up was prepared in triplicates.

Experimental Set-ups

300 μ L of the solution was spread and air-dried on a pre-weighed glass slide. The lignin and the commercial UV-absorbance agents were set up in three environments: (1) unexposed,

(2) exposed to sunlight but covered with a *WRX* glass bottom, and (3) exposed directly to sunlight. The time of exposure was 3 hours (12:40H–15:40H).

The hour-by-hour UV index forecast of the USA area code of the study was taken from the US Environmental Protection Agency, and the temperatures were also noted (Table 1). The optical properties of the background used for the exposed samples are given in Table 2. The brightness and opacity were taken using the Technidyne Color Touch 2 Model ISO. The L, a*, and b* were measured using the TAPPI T 527 standard method.

Table 1
Hour-by-hour UV index forecast and temperature of the experimental set-up

Time	Temperature (°F/°C)	UV-Index Forecast*
12:00H	63/17.2	3
13:00H	63/17.2	4
14:00H	65/18.3	3
15:00H	66/18.9	3
16:00H	65/18.3	1

Table 2
Optical properties of the background paper of the exposed samples

Brightness	Opacity	L*	a*	b*
92.092	99.462	98.22	-0.43	2.49

UV-Vis Spectrophotometry

The collected slides were allowed to equilibrate with the room temperature before it was weighed. The exact mass of the samples was noted by weighing them by difference.

The samples were dissolved in a known volume of dimethyl sulfoxide (DMSO). The lignin samples were diluted to 33x while the oxybenzone and the octinoxate were diluted to 11x31x (or 341x) to fit the accuracy range of the UV-Vis Spectrophotometer. Data on the total area under the curve within the UV range (200–400 nm) were gathered. Area under the curve for UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm) were also noted.

Determination of the Lignin Absorption Characteristics

The wavelength where the maximum absorption for each lignin in the UV range was determined using the UV-Vis Spectrophotometry. The five lignin samples were oven-dried for 12 hours. An amount of 0.0250 g of lignin was dissolved in DMSO and diluted to mark in a 25 mL flask. It was further diluted, as shown in Table 3. It was done in triplicates. The absorbance was recorded at the maximum wavelength, and the absorptivity was calculated using the slopes of the absorbance-concentration curve. The relationship between the absorption capacity and absorptivity was also determined.

RESULTS AND DISCUSSION

UV Absorbance Spectra

The absorbance of the lignin, functionalized lignin, and the commercial sunscreen agents were taken within the UV range. Lignin samples displayed maximum peak

Table 3
Stock solution dilution table

Lignin	Stock Solution Volume (μL)	DMSO (μL)	Concentration (mg/mL)
PL	240	2760	0.08
SMRecL	210	2790	0.07
WRecL	180	2820	0.06
WAc	150	2850	0.05
WFAE	120	2880	0.04

(λ_{max}) at 260-280 nm (Figure 1). λ_{max} is a characteristic absorption band of aromatic and polyphenolic structures (Lagesson et al., 2000). On the other hand, octinoxate has a relative peak at ~255 nm, which is attributed to benzene rings, but its λ_{max} is at 311nm, which is due to its anisole moiety (Hopkins et al., 2017; Kafle, 2020; Sambandan & Ratner, 2011). Oxybenzone has peaks at ~260 nm due to the presence of aromatic rings and has λ_{max} at ~290 nm, which is attributed to phenols (Sambandan & Ratner, 2011). Figure 2 (a-g) show the UV absorption spectra of octinoxate and oxybenzone.

Radiation-enhanced UV Absorption Effect

The photostability of lignin is an indirect measure of its efficiency even after long exposure to UV radiation. Octinoxate is known to degrade after a certain period of exposure (Sambandan & Ratner, 2011), which is evident by the observed color change discussed in the succeeding subtopic. UV-Vis Spectrophotometry showed that its UV absorption capacity was reduced by 7.95% after 3 hours of exposure to sunlight. UV absorption spectra in Figure 2 not only

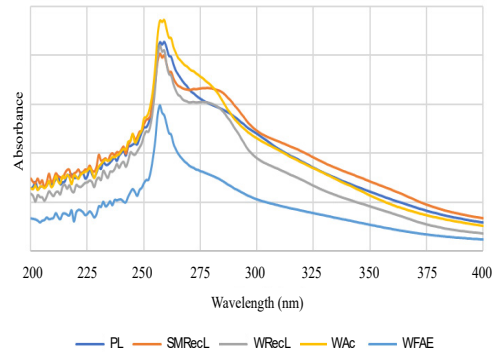


Figure 1. UV absorbance spectrum of unexposed lignin normalized to absorbance of 1 mg/mL of the substance

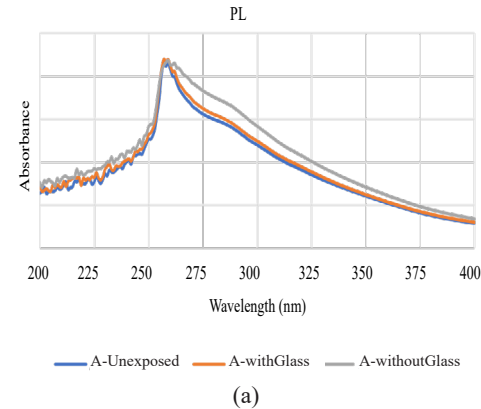


Figure 2. UV absorbance spectra of lignin derivatives and commercial sunscreen agents normalized to the absorbance of 1 mg/mL of the substance

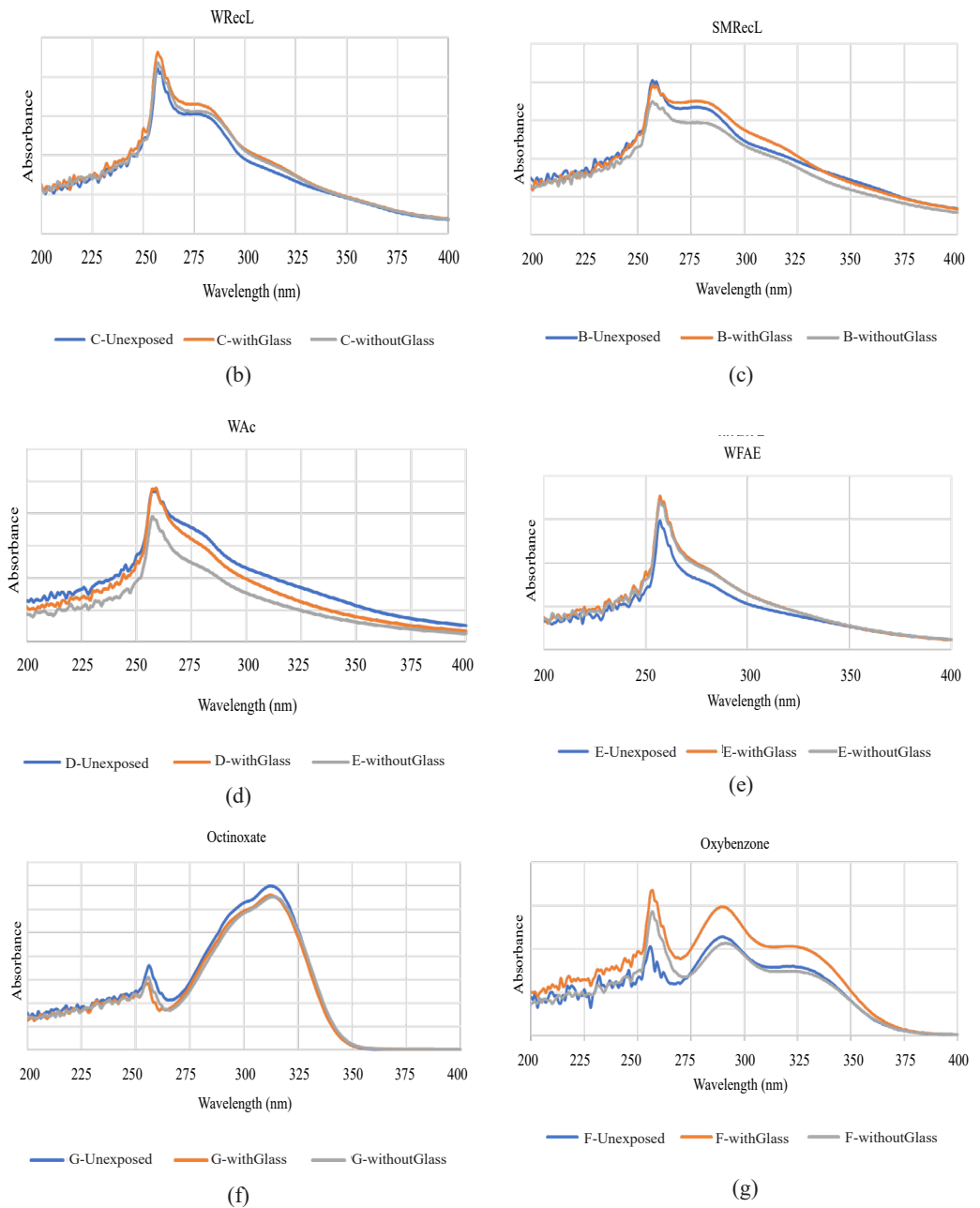


Figure 2. Continue

prove the photostability of lignin but also show that its UV absorbance increases, especially in the UVB region, after exposure to sunlight. For example, the UV absorbance capacity of PL and WFAE increased by 13.31% and 12.60%, respectively. It is true for almost all the samples of lignin except for WAc. The same results were obtained by Qian et al. (2015), who described this observation as “unexpected and promising.” Although the exact mechanism is not yet understood, it may be attributed to lignin's free radical scavenging ability due to its wide variety of functional groups and phenylpropanoic units. Not many available studies have explored this phenomenon, thus opening more interesting scientific inquiries.

Among WRecL, WAc, and WFAE, unmodified willow lignin (WRecL) is the most photostable. WAc has a slight decrease in its absorption intensity after exposure to sunlight. The blocked hydroxyl and methoxyl groups are expected to enhance the aromatic rings' UV absorption capacity (Figure 3). This trend was also expected for the WFAE; however, ^{31}P -NMR of this sample shows that the amount of carboxylic acid groups is high in this esterified lignin. The carboxylic groups form a conjugated system with aromatic structures that stabilize the UV absorption capacity (Ovadias, 2019; Young et al., 2017).

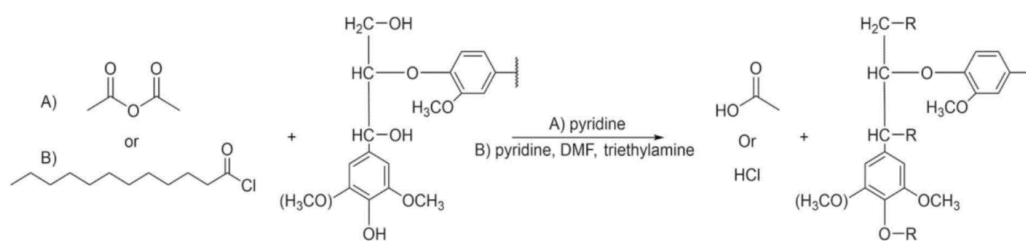


Figure 3. Proposed acylation of β -O-4 lignin structure by A) acetylation (C_2 addition) or B) acylation with lauroyl chloride (C_{12} addition)

Source: Nagardeolekar (2020)

Color stability is very important in marketing cosmetics and pharmaceutical products. Although lignin has a dark color that obstructs its usage in commercial products, a study by Zhang et al. (2019) showed that it can be whitened through fractionation and follow-up acetylation. Afterward, it can still be used effectively in sunscreen applications. Contrasting this potential of lignin, Figure 4 shows the yellowing of octinoxate after 3 hours of exposure to sunlight. Nanoparticle encapsulation improves the photostability of octinoxate (Perugini et al., 2002), yet it degrades upon exposure to sunlight and decreases its efficiency (Sambandan & Ratner, 2011).

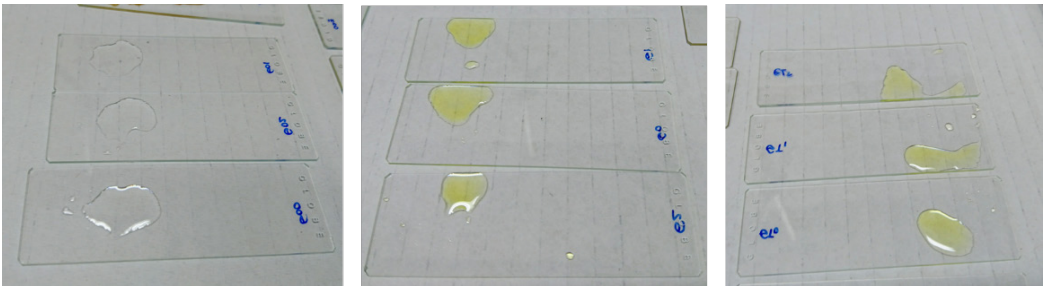


Figure 4. Yellowing of octinoxate upon exposure to sunlight. (Left to right: unexposed, exposed with glass cover, exposed directly to sunlight)

Absorption Characteristics

The absorbance and the concentration follow a linear relationship, as indicated by Beer-Lambert’s Law). Absorption intensity can be expressed as absorbance according to Equation 1:

$$Absorption\ (A) = \log \frac{I_0}{I} = kcb = \epsilon cb \qquad [1]$$

where k is a constant of solute, c is the solute concentration, b is the path length through the sample, and ε is a constant called absorptivity. The absorptivity coefficients for the lignin are presented in Table 4. PL and SMRecL have the highest absorptivity values among the lignin, while WFAE has the lowest. Therefore, PL and SMRecL will exhibit the highest absorbance ability, while WFAE will have the lowest given the same concentration. The linear relationship between the absorbance and the absorptivity can be extended to the total absorption capacity and the absorptivity. Figure 5 illustrates this relation.

Total UV Absorption Capacity

On average, the total absorption capacity of the lignin samples is lower by 6.4x and 16.3x compared to oxybenzone and octinoxate, respectively (Figure 6). Although this is the

Table 4
Absorption characteristics of the selected crude and functionalized lignin

Sample	Wavelength at Maximum Absorption (λ _{max}) [nm]	Absorptivity [L*g ⁻¹ *cm ⁻¹]
PL	261	19.488
SMRecL	279	19.057
WRecL	278	16.292
WAc	268	16.078
WFAE	259	9.447

case, the direct linear relationship between the concentration and the total absorption capacity, as presented in Figure 5, proves that it can be compensated by increasing the lignin concentration. Increasing the lignin concentration in the sunscreen can also increase the total absorption capacity. It can also be used synergistically with other sunscreen agents to lower the amount of relatively unsafe ingredients. Zhang et al. (2019) found that blending 8% light-colored

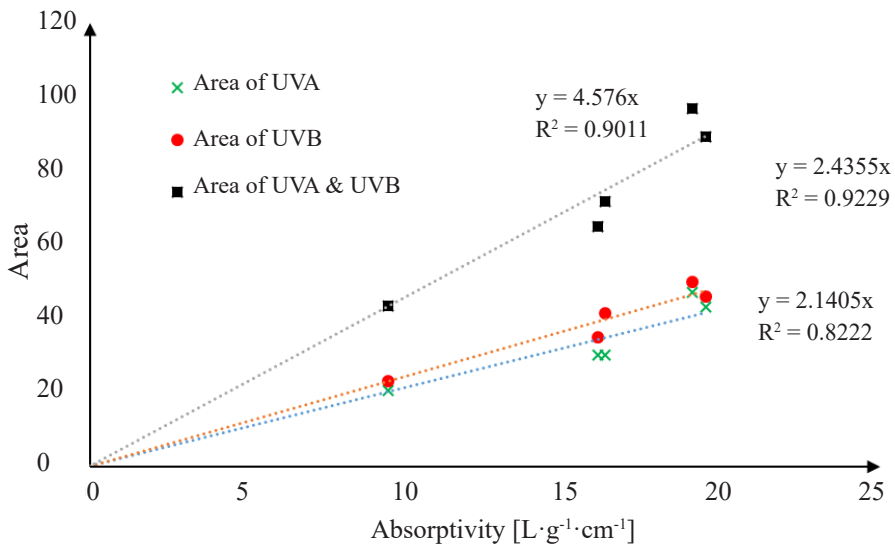


Figure 5. Relationship between absorption capacity and absorptivity

lignin with an SPF 15.3 lotion improved the SPF to 75.2. It proves lignin's potential in increasing sunscreens' UV absorption capacity.

The absorptivity coefficients and the total absorption capacities of the PL and SMRecL show no significant difference. No definite conclusion can be made since these lignin came from different species and processes. However, comparing the absorptivity coefficients and total absorption capacities of SMRecL and WRecL, WRecL showed a slightly lower capacity to absorb UV. The functionalized lignin shows a relatively lesser UV absorption capacity than the other. It may be due to the blocked hydroxyl and methoxyl groups that could have enhanced the UV absorption capacity of the aromatic rings.

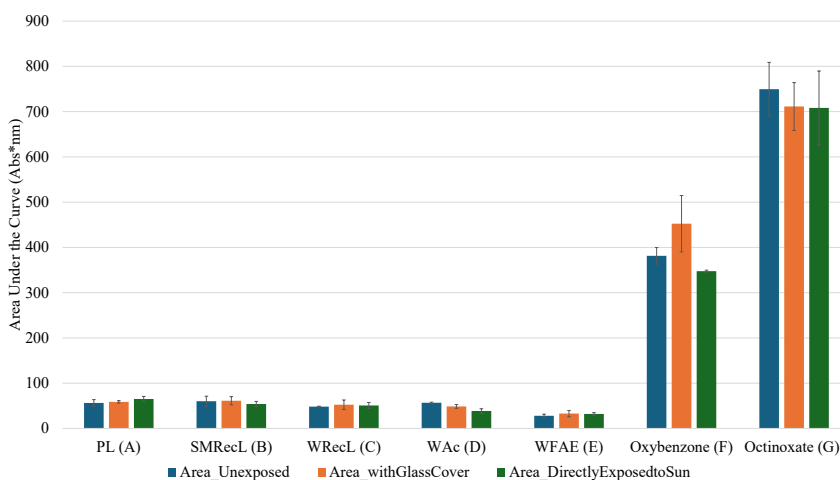


Figure 6. Total UV absorption capacity of lignin derivatives and commercial sunscreen agents normalized to a concentration of 0.1 mg/ 33 mL (33x dilution)

Broad-spectrum UV Absorption Capacity

One of the fundamental requirements for sunscreen is broad-spectrum coverage; that is, it covers most of the entire UV range, especially the UVA and UVB areas (Sambandan & Ratner, 2011). The absorption of the lignin and functionalized lignin samples were evaluated in the three UV regions. Results illustrated in Figure 7 show the potential of lignin in broad-spectrum sunscreen applications. Results showed that at a dilution of 33x, lignin and modified lignin absorbs up to more than 30 (absorbance unit-nm) in the UVA and UVB regions. These capacities can, of course, be intensified by increasing the concentration of lignin or by mixing it with other UV-absorbing agents.

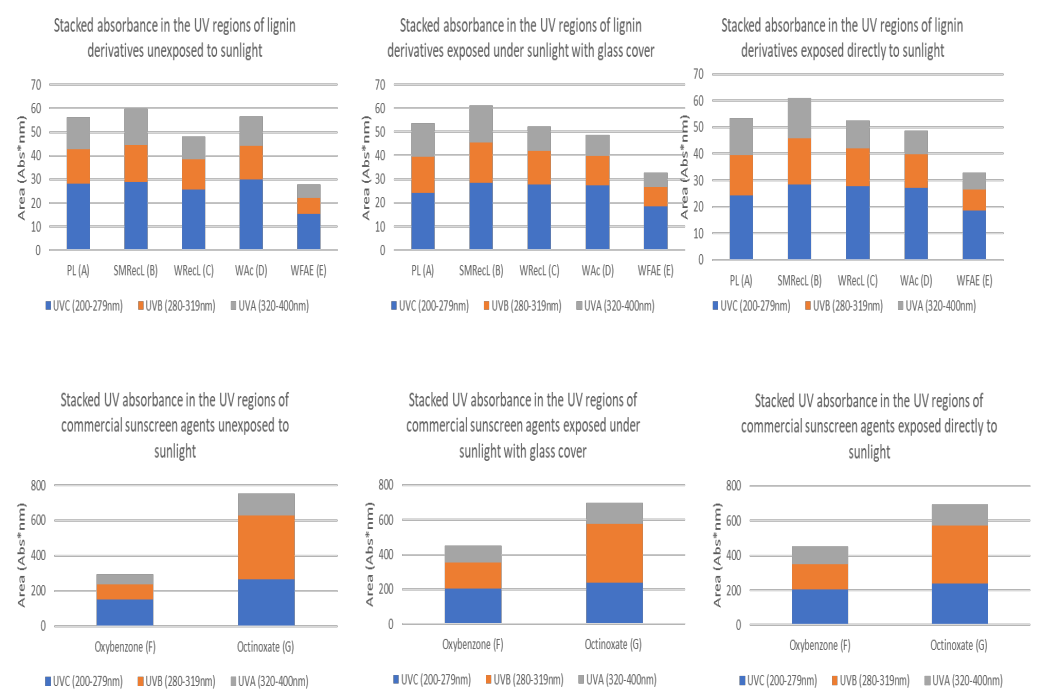


Figure 7. Stacked absorbance in the UV region of lignin and commercial sunscreen agents (normalized to a concentration of 0.1 mg/ 33 mL or 33x dilution)

CONCLUSION

UV absorption spectra of lignin and modified lignin samples were taken and were compared with commercial sunscreen agents—oxybenzone and octinoxate. Absorption peaks occurred at ~255–280 nm, reflecting the aromatic and polyphenol structures of the compounds. Unexpected results showed that most lignin samples displayed increased UV absorption capacity after sunlight exposure, which could be attributed to lignin's free radical scavenging ability. Lignin samples showed good color stability and photostability;

in contrast, yellowing of octinoxate was observed after 3 hours of sunlight exposure. Lignin has a lower UV absorption capacity compared to octinoxate and oxybenzone. However, it can be compensated by increasing its concentration or mixing it with other agents to synergistically increase its UV absorption capacity. Lignin also covers a good range of UVA and UVB regions and thus has a great prospect as an ingredient to natural broad-spectrum sunscreen lotions.

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SUPPLEMENTARY DATA

Extraction and Processing of the Lignin

Pure Lignin (PL) (Pure Lignin Environmental Technology Ltd, 2010)

The lignin was obtained from the #9 and #10 production line of Pure Lignin Environmental Technology Ltd processing of dry and green pines, respectively. The lignin is collected using gravity filtration. Five hundred grams of the lignin was washed with 500 mL of water (pH 6.63) and 0.30 ml of sulfuric acid (pH 2.05). The first of four-reverse osmosis (RO) water (250 mL, pH 6.65) washing was done when a thin film of the acid wash appeared on top of the lignin.

Sugar Maple Recovered Lignin (Dongre, 2018)

The sugar maple (*Acer saccharum*) chips were subjected to hot-water extraction (HWE) under 160°C for 2 hours. HWE was carried out in a Struthers-Wells 65 ft³ stainless-lined batch digester. The water-bust biomass ratio was 4. The extract from HWE underwent ultrafiltration through a Hilco HM634-01 ceramic filter with a pore size of 0.01 µm. The retentate was recovered, and the pH was adjusted to 2 before centrifugation to collect the sugar maple fraction. It undergoes nanofiltration, and the permeation is acid hydrolyzed with concentrated sulfuric acid (1.5% by mass of extract) at 130°C for 45 min. The precipitate was recovered with dissolution in acetone/water (1:1) binary solvent and steam-stripped to remove the solvents.

Willow Recovered Lignin (WRecL) (Ovadias, 2019)

The hydrolysates of recovered willow (*Salix spp.*, Family: Salicaceae; a blend of commercial cultivars with bark) were from the pilot plant biorefinery in PBE, SUNY-ESF. The pH of the hydrolysate was dropped to 2 using 20% H₂SO₄, and the lignin was precipitated and collected through centrifugation and vacuum filtration.

Acetylated Willow Lignin (WAc) (Ovadias, 2019)

WRecL was dissolved into 20 mL/g-lignin of pyridine and acetic anhydride (1:1 v/v). The solution was mixed on a stir plate for at least 24 hours and up to 72 hours. 300 mL ice-cold water per one gram of starting lignin was poured to precipitate the acetylated lignin. It was collected using vacuum filtration and again washed with ice-cold water to remove excess pyridine, acetic anhydride and acetic acid (a byproduct of the reaction). The acetylated lignin was dried overnight in a vacuum oven (40°C). When scaling this reaction up to 5 grams, 70 mL of pyridine and acetic anhydride was utilized (0.4:1 v/v) to reduce pyridine usage. Appendix Figure A1 shows the proposed reaction mechanism.

Willow Fatty Acid Esterified (WFAE) (Ovadias, 2019)

WRecL was acylated with lauroyl chloride, a chloride derivative of lauric acid, by dissolving into 30 mL of N, N-dimethylformamide per gram of lignin in the presence of

pyridine (5.5 mL) and triethylamine (1.5 mL). Once the solution was homogenized, 1 mmol-lauroyl chloride/mmol-OH was added to start the reaction. Constant stirring was employed for 2 hours at room temperature. The lignin acylated with lauroyl chloride was precipitated by pouring the solution into 600 mL of ice-cold 2% HCl per one gram of starting lignin. The mixture was vacuum-filtered with nylon filters to make it easier to recover the lignin laureate. The filter cake was then washed with ethanol and water (1:1 v/v) to remove excess solvent, unreacted lauroyl chloride, and unbound fatty acid, lauric acid. The lignin laurate was dried overnight in a vacuum oven (40°C).