KIMIKA Volume 32, Number 2, pp. 1-10 (2021)
 ISSN 2508-0911
https://doi.org/10.26534/kimika.v32i2.1-10

Extraction of Milled Wood Lignin from Coffee Husk (*Coffea arabica* L.) and the Analysis of Its Potential as a UV-Protective Component of Lotion and Sunscreen

Christian John G. Cordero and Kevinilo P. Marquez*

Institution of Chemistry, College of Arts and Science, University of the Philippines Los Baños, College, Laguna, 4030

*Author to whom correspondence should be addressed; email: kgperez3@up.edu.ph

ABSTRACT

This study aims to determine the effect of adding milled wood lignin (MWL) to the sun protection factor (SPF) of commercially available lotion and sunscreen. The MWL was isolated from *Coffea Arabica* L. husks using the Björkman method. FT-IR spectroscopic analysis showed two characteristic peaks located at around 1200 cm^{-1} and 1300 cm^{-1}, which indicates the presence of guaiacyl and syringyl groups. UV-Vis spectroscopic analysis showed two peaks located at around 278 nm and 325 nm, which indicates the presence of guaiacyl and some conjugated phenolic groups, respectively. The isolated MWL was then added to commercially available sunscreen and lotion at various concentrations. The sun protection factor (SPF) was then determined using the Mansur method and UV-Vis Spectroscopy. Results have shown that from 0% to 40 % by mass of MWL added, there was an observable average increase from 0.90 to 13.86 SPF for lotion, while there was an increase from 26.47 to 34.00 SPF for sunscreen. Results suggest that for lotion the SPF significantly increased if MWL was added from 0% up to 40% while for sunscreen the SPF significantly increased if MWL was added from 0% up to 20%.

Keywords: *Björkman method, Mansur method, Milled wood lignin, sun protection factor*

INTRODUCTION

Skin aging (such as skin spots and wrinkles), sunburn, and skin cancer, one of the most common of all cancers can be caused by exposure to the sun's ultraviolet (UV) radiation (Linos et al., 2016). Solar radiation is a genotoxic agent; it damages the DNA, which then results in cellular mutations that are generally harmful to the body. Since UV radiation is the most harmful mutagenic radiation (Wang, 1976), it is recommended by the US Food and Drug Administration (FDA) and the American Academy of Dermatology (AAD) to use sunscreen even in broad-spectrum cloudy days (Gabard, 1999).

There are two types of active ingredient in sunscreens – organic and inorganic. The difference, aside from their structure, is on their sun-protection mechanism (Green et al., 2011). Current commercial products use various combinations of organic and inorganic sunscreens to broaden the absorbance spectrum in the UV region (Gasparro et al., 1998), but the preparation for these substances is generally costly. This study offers the possibility of using lignin as a cheaper alternative to these substances.

Lignin (Figure 1) is a heterogeneous and phenolic polymer (Lebo et al., 2001), which can be found in most terrestrial plants and is very abundant in plants, being the second to the most abundant renewable resource on earth, next to cellulose. It has structural features that can be a potential source of useful chemicals such as UV blocking agents, antioxidants, antimicrobial compounds, as well as adsorbents for heavy metal and organic contaminants (Marquez and Ramos, 2019). It contains mostly aromatic groups, like the guaiacyl group (Figure 2a) and syringyl groups (Figure 2b). Aside from the abundance of UV-absorbing phenolic groups in its structure, lignin and other lignin-based products exhibit low cytotoxicity (Freitas, et al, 2020).

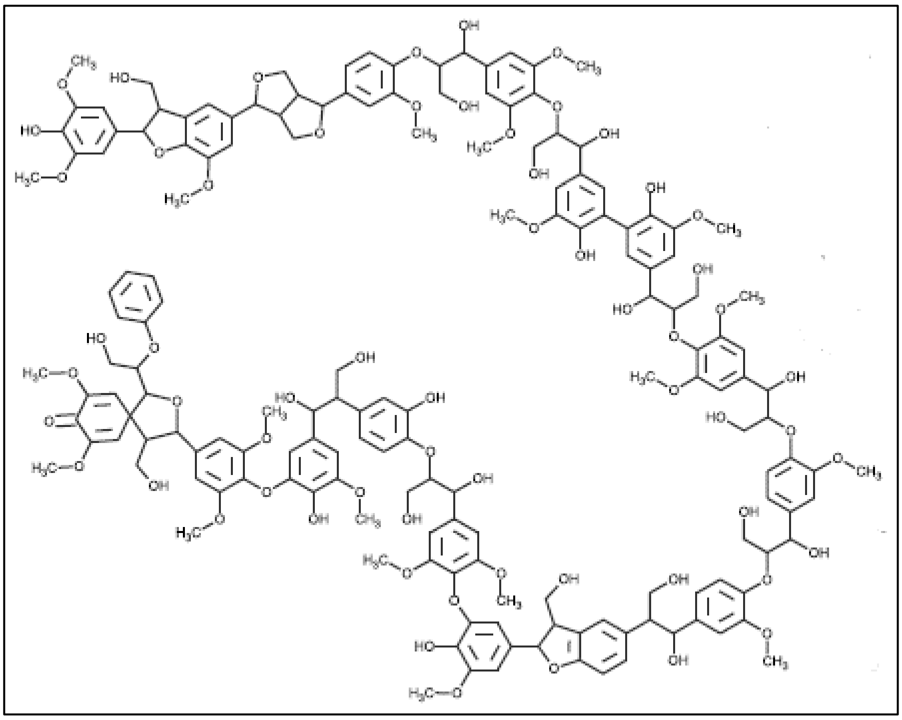

Figure 1. Schematic representation of a lignin structure (Yáñez-S, et al., 2014)

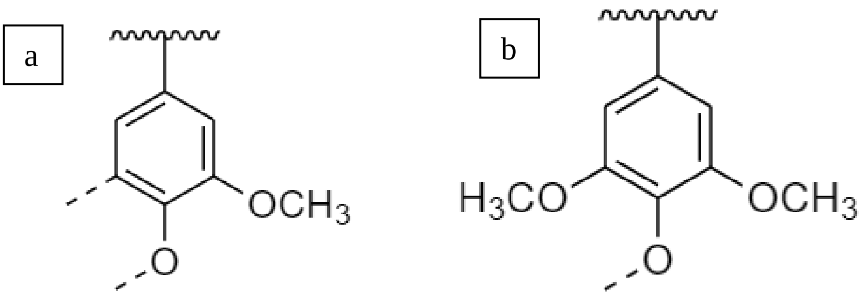

Figure 2. Structures of (a) Guaiacyl group and (b) syringyl group

Coffee husk is considered as one of the major contributors in Philippine agricultural wastes, with an annual average production of 30,000 metric tons. Its availability and low cost make it a potentially economical source of lignin. Milled Wood Lignin (MWL) was isolated from the husk of *Coffea arabica* L. and was analyzed for its potential use as additive in sunscreen. MWL is colorless, unlike kraft, soda, and organosolv lignin making it an ideal additive as it does not impart coloration to the product. The chromophores that are responsible for the dark color are introduced into lignin during isolation, and it can be accelerated by harsh conditions (e.g., high temperature and very low pH). Thus, to minimize the coloration of the lignin product, the extraction must be done at milder conditions. Moreover, methods that involve components like sulfides, strong acids, strong bases, and some organic solvents may not be suitable for its purpose, unless otherwise a very stringent purification method is employed after extracting lignin. This additional task might further decrease the yield of extracted lignin.

In this study, the sun protection ability of MWL isolated from the husk of *Coffea arabica L.* was explored as an additive to sunscreen and lotion. We report herein the incorporation of MWL in commercial skincare products and its ability to block various wavelengths of UV radiation was analyzed by determining the Sun Protection Factor (SPF) through its absorption at various wavelengths in the UV region.

METHODOLOGY

Materials and Equipment. Reagents were purchased from Chemline Scientific Inc. and used as received. The *Coffea arabica L.* husk samples were obtained from the Agronomy Department in UPLB. The samples were authenticated in the UPLB Museum of Natural History, College of Forestry and Natural Resources, UP Los Baños. The husks were sun-dried to remove the moisture before being cut into smaller pieces. The lotion and sunscreen were commercially available products obtained from local stores. For the UV-Visible (UV-Vis) Spectrometry analysis, Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer from Thermo Fisher Scientific was used. For ATR-FTIR analysis, IRSpirit Fourier transform infrared (FTIR) spectrometer from Shimadzu was used. The lotion that was used in this study is NIVEA Moisturizing Cream and the sunscreen lotion was Nivea Sun Protect and Moisture SPF 30.

Lignin Preparation. Lignin was isolated from *Coffea arabica L.* based on the method of Björkman (Björkman, 1954). In this procedure, the dried coffee husk was washed with ethanol to remove impurities. It was then extracted twice with 96:4 dioxane:water ratio by volume for 48 hours with constant stirring. The extract was then dried using rotary evaporator (40°C) to afford the MWL.

Characterization of Lignin. Isolated lignin from *Coffea arabica L.* was characterized using UV-Vis and ATR-FTIR Spectroscopies. The dried isolated lignin was first dissolved in 96:4 dioxane:water (0.2mg/L) then, the absorbances between 250 nm to 400 nm at 1 nm intervals were obtained. For ATR-FTIR analysis, small amount of dried isolated lignin was mounted on ATR-FTIR machine.

Determination of the Sun Protection Factor (SPF). The method was adapted from the studies of Dutra, et al. (2004), and of Lin and Dence (1992).

UV absorbance measurements for lotion and sunscreen. One gram of each sample was obtained and were then diluted to 100-mL volumetric flasks with ethanol. Then, the samples were degassed for 5 min and each were filtered through filter paper. Then, a 5 mL aliquot for each sample was diluted to 50-mL volumetric flask with ethanol. Then, 5 mL of the diluted aliquot was obtained and transferred to 25-mL volumetric flask. The solutions were then stored in glass vials. The experiment was prepared at room temperature and in a dark room to minimize stray light (Lee et al., 2019).

The absorbances of the solutions were then obtained using the UV-Vis spectrophotometer, obtained between 290 nm to 320 nm at 5 nm intervals. The analysis was done in three replicates for each sample. The Mansur equation was then applied to calculate the SPF values based on the absorbances obtained (Mansur et al., 1986):

$$SPF\ (spectrometry) = CF\ \times \sum_{290}^{320} EE\ (\lambda) x\ I\ (\lambda)\ x\ Abs(\lambda) \qquad (1)$$

where EE (λ): Erythemal effect spectrum; I (λ): Solar intensity spectrum; Abs (λ): Absorbance of sunscreen product; CF: Correction factor. The normalized values of EE (λ), I (λ), and CF are constants that are dependent on the wavelength of light and are already determined in the published 1986 study of Mansur et al.

UV absorbance measurements for lotion and sunscreen with added MWL. The isolated lignin solution was added to pure lotion and the sunscreen solutions. The concentrations of lignin in each of the solutions were 10% (0.02mg/mL), 20% (0.04mg/mL), 30% (0.06mg/mL), and 40% (0.08mg/mL).

The lignin was dissolved first in dioxane (1mg/L) then added to the lotion and sunscreen samples in different concentrations. One gram of each mixture was then obtained and diluted to 100 mL with ethanol, degassed for 5 min, and then filtered through filter paper. Then, a 5 mL aliquot was obtained for each sample and diluted to 50 mL with ethanol. Then, 5 mL of the diluted aliquots were obtained from each sample and were further diluted to 25 mL with ethanol. The solutions were then stored in glass vials. The whole process was done at room temperature in a dark room to minimize stray light. The solutions were then stored in quartz vials.

The samples were then subjected to UV-Vis spectrophotometric analysis between 290 nm and 320 nm at 5-nm intervals, where the absorbances at these wavelengths were recorded. Analysis for each sample was done in three (3) replicates. The Mansur equation was applied to calculate SPF values.

Testing of significance. One-way ANOVA and T-tests were done to determine if there was a significant difference between the SPF values of the pure lotion and sunscreen solutions to those with added lignin, as well as comparisons between those with different amounts of added lignin. All statistical analyses were performed using GraphPad Prism 8.3.0 software.

RESULTS AND DISCUSSION

Isolation of Milled Wood Lignin (MWL). Table 1 shows the results of extracting the MWL from coffee husk. The yields are expected, since the *Coffea arabica* L. husks typically contain about 20%-30% lignin by mass (Obst & Kirk, 1988).

Table 1. Data on the results of MWL extraction using the Björkman method.

Trials	Mass of the Sample, g	Mass of Extracted MWL, g	Theoretical Yield, Based on 30% lignin content (Obst & Kirk, 1988), %	Experimental Yield, Based on 30% lignin content (Obst & Kirk, 1988), %
Trial 1	20.0315	0.8194	6.00945	13.6352
Trial 2	20.0293	0.7871	6.00879	13.0991
Trial 3	20.2044	0.9603	6.06132	15.8431
Average	20.0884	0.8556	6.02652	14.19247

The color of the isolated MWL from coffee husk (Figure 3) were slightly darker than what was expected from the typical color of MWL. This may be due to the dark color of the sample.

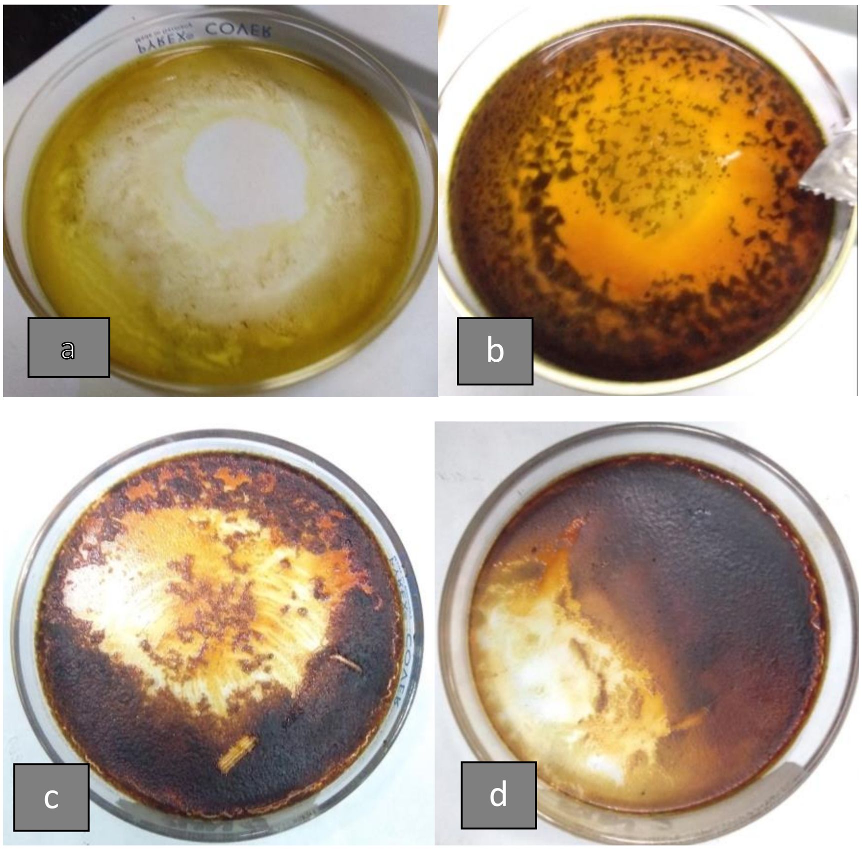

Figure 3. Milled Wood Lignin (a) Preliminary Trial, (b) Trial 1, (c) Trial 2, (d) Trial 3

Characterization of the isolated lignin. The characterization of the isolated lignin was done using UV Vis spectroscopy and ATR-FTIR Spectroscopy.

ATR-IR Spectroscopy of the Isolated Lignin. The results for the ATR-IR Spectroscopy of MWL of Coffee Husk shown in Figures 4a, 4b, and 4c, while Table 2 shows the assignments of ATR-IR spectra. All of them show a broad peak at around 3350 cm^{-1} which indicates the presence of hydroxyl groups. There are two medium-to-strong peaks at around 2950 cm^{-1} and 2850 cm^{-1}, which indicates presence of the aliphatic group. Carbonyl groups are also present due to the strong peaks in the 1700 cm^{-1} and 1600 cm^{-1} regions, which indicates presence of the unconjugated and conjugated carbonyl groups, respectively. Presence of the conjugated carbonyl groups indicate the presence of the coumaryl ester group. The peak at around 1350 cm^{-1} is due to the syringyl group, while the peak at around 1250 cm^{-1} is due to the guaiacyl group. Figure 4d shows the FT-IR spectra of milled wood lignin in literature (El Hage et al., 2009) and Table 2 also shows the assigned values for the spectra. The broad band around 3435 cm^{-1} represents the hydroxyl (-OH) stretch. The medium to strong peaks at the 2800 cm^{-1} to 3000 cm^{-1} region represents the aliphatic group. Carbonyl group is one of the main functional groups in the lignin structure. The bands of this functional group lie between 1760 cm^{-1} and 1650 cm^{-1}. There are two strong bands observed at these regions attributed to the unconjugated carbonyl and conjugated carbonyl stretch at 1709 cm^{-1} and 1660 cm^{-1}, respectively. The two important peaks are in the 1200 cm^{-1} and 1300 cm^{-1} which are the guaiacyl and syringyl groups, respectively (El Hage et al., 2009).

Table 2. Assignment of ATR-IR Spectra of Milled Wood Lignin of Coffee Husk.

Peaks (cm^{-1})				Assignment
Trial 1	Trial 2	Trial 3	Lignin from *Miscanthus* (El Hage et al., 2009).	
3350	3330	3330	3435	OH-stretching
2950	2950	2920	2918	Aliphatic groups (alkane)
2850	2820	2820	2800	Aliphatic groups (alkane)
1700	1700	1700	1709	C=O stretch (unconjugated)
1650	1650	1650	1660	C=O stretch (conjugated)
1550	1545	1550	1508	Aromatic squel vibration
1450	1450	1450	1422	Aromatic ring vibration
1355	1350	1360	1328	Syringyl ring breathing, C–O stretch
1225	1240	1240	1267	Guaiacyl ring breathing

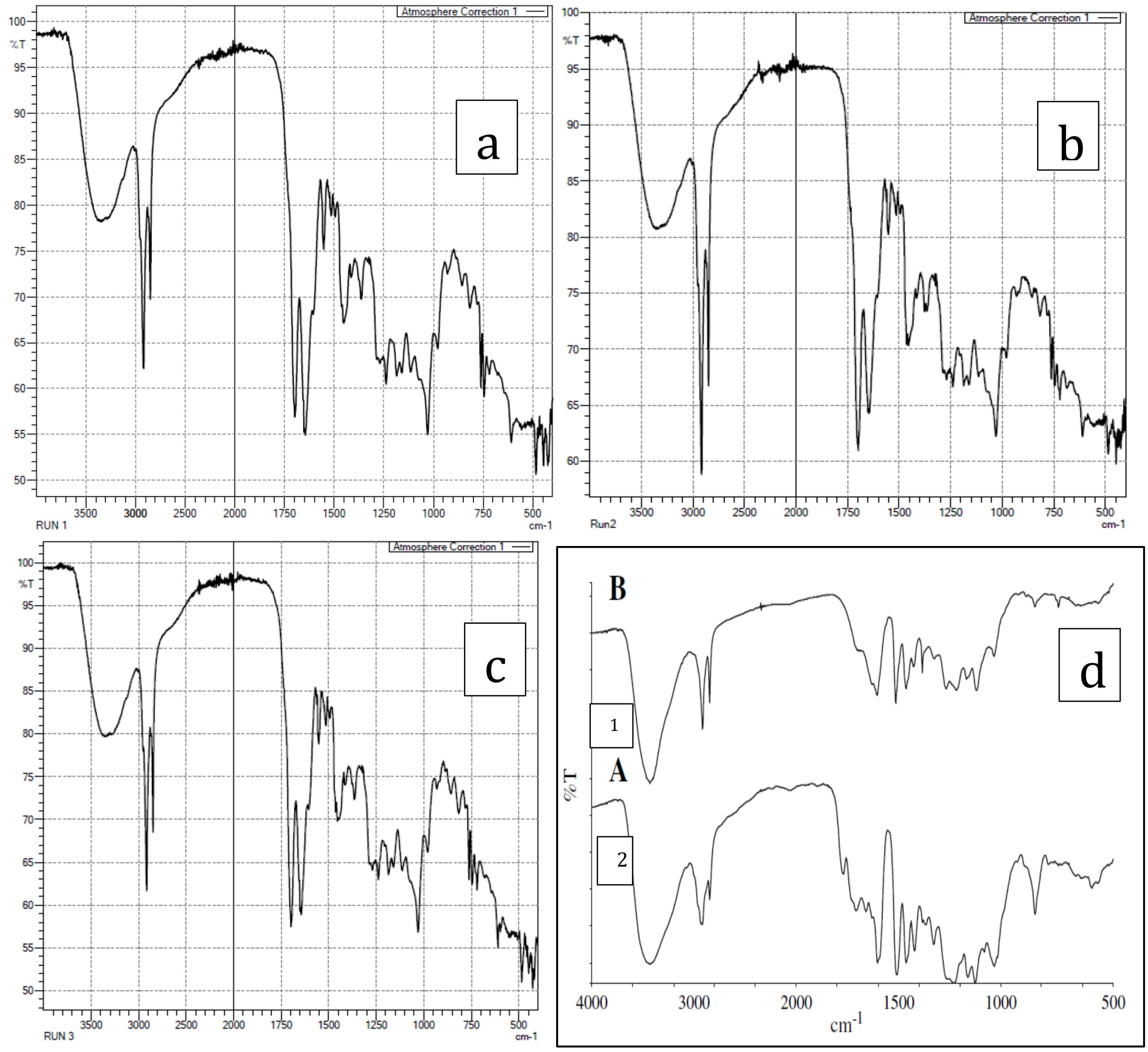

Figure 4. ATR-FTIR Spectra of Milled Wood Lignin of Coffee Husk a. TRIAL 1, b. TRIAL 2, c. TRIAL 3, d. ATR-FTIR Spectra of milled wood lignin (1) and ethanol organosolv lignin (2) isolated from *Miscanthus* (El Hage et al., 2009).

UV-Vis Spectroscopy of the Isolated Lignin. The result for the UV-Vis spectroscopy of the MWL of coffee husk is shown in Figure 5a and the UV-Vis spectra of the MWL in the literature is shown in Fig. 5b (El Hage et al., 2009). The two peaks in the spectra of MWL was due to the presence of non-conjugated and conjugated phenolic groups, such as the p-coumaric and ferulic acids (Xu et al., 2007). The first peak at 282 nm indicates the presence of guaiacyl-rich lignins. The second peak at 315 nm indicates the presence of conjugated phenolic groups like the coumaryl ester group. In Figure 5a, the three have relatively similar spectra. The two peaks represent the absorbance of conjugated and unconjugated phenolic groups (El Hage et al., 2009; Sayre et al., 1979). Trial 1 has a maximum absorbance at 276 nm and another peak at 324 nm. Trial 2 has a maximum absorbance at 277 nm and another peak at 325 nm. Trial 3 has a maximum absorbance at 278 nm and another peak at 325 nm.

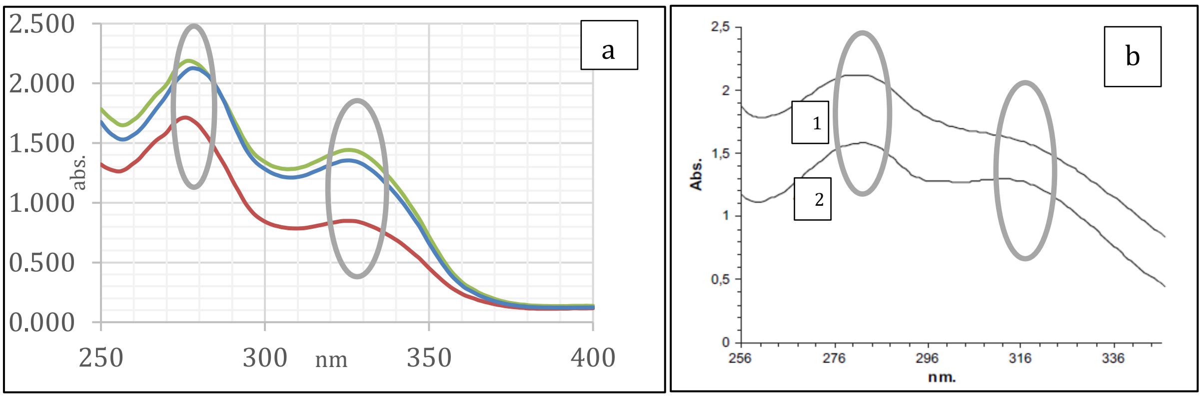

Figure 5a. UV-Vis spectra of Milled Wood Lignin from Coffee Husk for Trial 1 (red), Trial 2 (green), and Trial 3 (blue); Prominent peaks are encircled in gray. 5b. UV-Vis spectra Milled Wood Lignin (1) and Ethanol Organosolv Lignin (2) isolated from *Miscanthus* (El Hage et al., 2009).

Sun Protection Factor Determination of Lotion with added MWL. The MWL from coffee husk was mixed in different concentrations with lotion. Table 3 shows the obtained SPF values for each formulation.

Table 3. Summary table for the determination of SPF of lotion with different lignin concentrations.

Concentration	TRIAL 1	TRIAL 2	TRIAL 3	Average	Std dev
0% lignin	1.26	0.44	1.01	0.90	0.42
10% lignin	4.38	3.31	3.48	3.72	0.56
20% lignin	8.41	6.55	7.10	7.35	0.96
30% lignin	11.46	10.24	10.14	10.61	0.73
40% lignin	15.08	13.50	12.99	13.86	1.09

To test the significance of adding lignin to the resulting SPF of the mixture, statistical tests of significance were performed. Based on the results of the T-Test and ANOVA, increasing the percentage of lignin significantly increases the SPF of the lotion up to 40% lignin.

All the data points per treatment were subjected to Grub's Test, where no outliers were obtained. The conclusions are presented in Table 4. Based on ANOVA, all the treatments were significantly different from each other. All the analysis results were statistically significant at $p < 0.05$.

Table 4. Statistical Significance in the SPF Differences between Various Formulations of Lotion based on Pairwise T-Tests at p < 0.05*

Concentration	Difference in SPF (Significant or Not Significant)				
	0% lignin	10% lignin	20% lignin	30% lignin	40% lignin
0% lignin	-	Significant	-	-	
10% lignin	Significant	-	Significant	-	-
20% lignin	-	Significant	-	Significant	
30% lignin	-	-	Significant	-	Significant
40% lignin	-	-	-	Significant	-

*T-Tests for regions in blank (-) were not performed

Sun Protection Factor Determination of Sunscreen with added MWL. MWL was mixed in different concentrations with sunscreen. Table 5 shows the obtained SPF values for each formulation. Figure 6 shows the plot of the resulting mixture's SPF versus the % added lignin.

Table 5. Summary table for the determination of SPF of lotion with different lignin concentrations.

Concentration	TRIAL 1	TRIAL 2	TRIAL 3	Average	Std dev
0% lignin	24.12	28.16	27.12	26.47	2.10
10% lignin	30.08	30.63	31.28	30.66	0.60
20% lignin	32.61	33.02	33.43	33.02	0.41
30% lignin	34.48	34.48	34.27	34.41	0.12
40% lignin	33.41	34.22	34.38	34.00	0.52

The statistical significance between the SPF values were also determined using the T-Test and ANOVA. Results show that increasing the percentage of lignin significantly increases the SPF of the sunscreen up to 30% lignin. The SPF increase from 30% to 40% lignin is not statistically significant. Like the lignin-added lotion, this means that further addition of lignin after 40% may not significantly increase the SPF of the resulting mixtures.

All the data points per treatment were subjected to Grub's Test, where no outliers were obtained. The conclusions from the T-tests are presented in Table 6. Based on ANOVA, all the treatments were significantly different from each other, like that for the MWL-added lotion. All the analysis results were also statistically significant at p < 0.05.

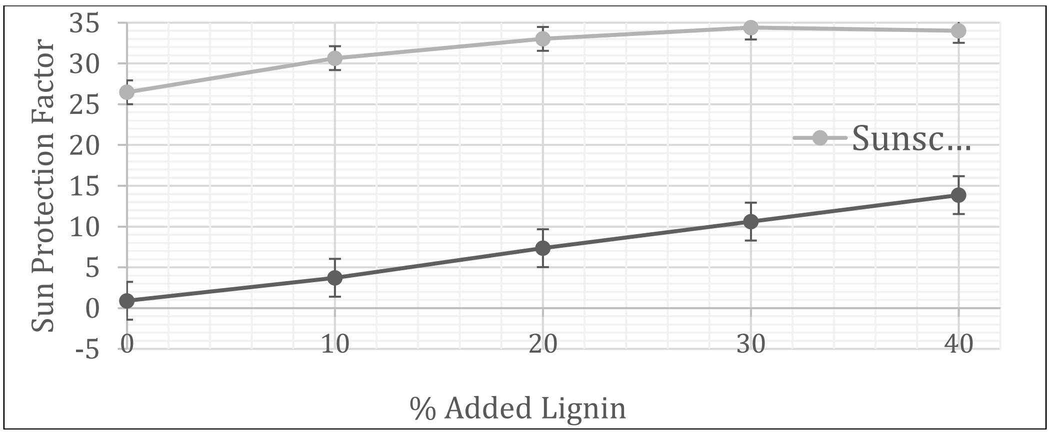

Figure 6. Plots of SPF versus % Added Lignin for both Lotion and Sunscreen

Table 6. Statistical Significance in the SPF Differences between Various Formulations of Sunscreen based on Pairwise T-Tests at p < 0.05*

Concentration	Difference in SPF (Significant or Not Significant)				
	0% lignin	10% lignin	20% lignin	30% lignin	40% lignin
0% lignin	-	Significant	-	-	
10% lignin	Significant	-	Significant	-	-
20% lignin	-	Significant	-	Not Significant	
30% lignin	-	-	Significant	-	Not Significant
40% lignin	-	-	-	Not Significant	-

*T-Tests for regions in blank (-) were not performed

CONCLUSIONS

Milled Wood Lignin (MWL) from *Coffea arabica L.* husks were extracted using the Björkman method, where the extracts were then characterized using UV-Vis and ATR-FTIR Spectroscopy. UV-Vis analysis showed prominent peaks due to the presence of conjugated and non-conjugated phenolic groups, both of which can be found in lignin. ATR-FTIR analyses confirmed the presence of hydroxyl and conjugated phenolic groups, which are indicative of lignin structural features. For the same purpose, it is recommended that other lignocellulosic waste be explored. Also, other extraction and purification methods can be explored for this purpose.

The potential of extracted MWL as an additive in lotion and sunscreen was explored in this study. Based on the results, addition of lignin until 40% significantly increases the SPF of the resulting mixture, but further addition may no longer increase the SPF to a significant degree. It is recommended, however, that other methods of SPF determination should be explored. The UV-absorbing properties of lignin may be affected by other factors, such as matrix effects and the presence of other components in the lotion and sunscreen. Significance in the SPF change for shorter % lignin intervals should also be explored to determine the extent of contribution of the lignin component to the overall SPF of the lotion or sunscreen.

REFERENCES

Björkman A. Isolation of lignin from finely divided wood with neutral solvents. Nature. 1954 Dec;174(4440):1057-1058. https://doi.org/10.1038/1741057a0

Dutra EA, Kedor-Hackmann ER, Santoro MI. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Revista Brasileira de Ciências Farmacêuticas. 2004 Sep; 40(3):381-385. https://doi.org/10.1590/S1516-93322004000300014

El Hage R, Brosse N, Chrusciel L, Sanchez C, Sannigrahi P, Ragauskas A. Characterization of milled wood lignin and ethanol organosolv lignin from miscanthus. Polym Degrad Stab. 2009 Oct; 94(10):1632-1638. https://doi.org/10.1016/j.polymdegradstab.2009.07.007

Freitas FM, Cerqueira MA, Gonçalves C, Azinheiro S, Garrido-Maestu A, Vicente AA, Pastrana LM, Teixeira JA, Michelin M. Green synthesis of lignin nano-and micro-particles: Physicochemical characterization, bioactive properties and cytotoxicity assessment. Int J Biol Macromol. 2020 Nov; 163:1798-1809. https://doi.org/10.1016/j.ijbiomac.2020.09.110

Gabard B. Sunscreens. In: Elsner P, Maibach HI, Merk HF, editors. Cosmetics: Controlled efficacy studies and regulation. Berlin, Heidelberg: Springer; 1999. https://doi.org/10.1007/978-3-642-59869-2_9

Gasparro FP, Mitchnick M, Nash JF. A review of sunscreen safety and efficacy. Photochem Photobiol. 1998 Sep; 68(3):243-256. https://doi.org/10.1111/j.1751-1097.1998.tb09677.x

Green AC, Williams GM, Logan V, Strutton GM. Reduced melanoma after regular sunscreen use: randomized trial follow-up. J Clin Oncol. 2011 Jan; 29(3):257-263. https://doi.org/10.1200/JCO.2010.28.7078

Lebo SEJ, Gargulak JD, McNally TJ. "Lignin," in Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc., 2001. https://doi.org/10.1002/0471238961.12090714120914.a01.pub2

Lee SC, Tran TM, Choi JW, Won K. Lignin for white natural sunscreens. Int J Biol Macromol. 2019 Feb; 122:549-554. https://doi.org/10.1016/j.ijbiomac.2018.10.184

Lin SY, Dence CW, editors. Methods in lignin chemistry. Springer Science & Business Media; 1992. https://doi.org/10.1007/978-3-642-74065-7

Linos E, Katz KA, Colditz GA. Skin cancer—the importance of prevention. JAMA Intern Med. 2016 Oct; 176(10):1435-1436. https://doi.org/10.1001/jamainternmed.2016.5008

Mansur JS, Breder MNR, Mansur MCA, Azulay RD. Determination of sun protection factor by spectrophotometry. An Bras Dermatol. 1986 May-Jun; 61:121-124. https://pesquisa.bvsalud.org/portal/resource/pt/lil-34224?lang=es

Marquez KP, Ramos RM. Alkali lignin from talahib grass (*Saccharum spontaneum* L.) as an adsorbent for chromium (III) and phenolphthalein: analysis of the adsorption kinetics and mechanism. KIMIKA. 2019 Dec; 30(2):17-26. https://doi.org/10.26534/kimika.v30i2.17-26

Obst JK, Kirk TK. Isolation of lignin. In: Wood WA, Kellogg ST, editors. Methods in enzymology – Biomass part B lignin, pectin, and chitin. San Diego, CA: Academic Press, Inc.; 1988. Vol 161, p 3-12. https://www.fpl.fs.fed.us/documnts/pdf1988/obst88b.pdf

Sayre RM, Agin PP, LeVee GJ, Marlowe E. A comparison of in vivo and in vitro testing of sunscreening formulas. Photochem Photobiol. 1979 Mar; 29(3):559-66. https://doi.org/10.1111/j.1751-1097.1979.tb07090.x

Wang SY, editor. Photochemistry and photobiology of nucleic acids. Elsevier; 1976. https://doi.org/10.1016/B978-0-12-734601-4.X5001-0

Xu F, Jiang JX, Sun RC, Tang JN, Sun JX, Su YQ. Fractional isolation and structural characterization of mild ball-milled lignin in high yield and purity from *Eucommia ulmoides* Oliv. Wood Sci Technol. 2007 Mar; 42(3):211-226. https://doi.org/10.1007/s00226-007-0162-5

Yáñez-S M, Matsuhiro B, Nuñez C, Pan S, Hubbell CA, Sannigrahi P, Ragauskas AJ. Physicochemical characterization of ethanol organosolv lignin (EOL) from *Eucalyptus globulus*: Effect of extraction conditions on the molecular structure. Polym Degrad Stab. 2014 Dec; 110:184-194. https://doi:10.1016/j.polymdegradstab.2014.08.026