Supporting Information

Development of a Light Activatable Lignin Nanosphere based Spray Coating for Bioimaging and Antimicrobial Photodynamic Therapy

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Materials and Instruments

Materials: Kraft lignin (Sigma Aldrich), potassium persulphate, fluorescein isothiocyanate (FITC), propidium iodide (PI), glutaraldehyde, 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), quercetin dihydrate, gallic acid, cetyl trimethyl ammonium bromide (CTAB), didodecyldimethylammonium bromide (DMAB), polyethylene glycol (PEG),

polyvinylpyrrolidone (PVP), pluronic F-68 (PF-68), Tween 80 were purchased from Sigma Aldrich. phosphate buffered saline (PBS), Luria broth, agar and agarose were procured from HiMedia Laboratories Pvt. Ltd. Deionized water was collected from the Merck Millipore system. The Gram negative bacterial strain *E. coli* (MTCC BL21) and Gram positive bacterial strain *B. megaterium* (MTCC 2444) were procured from microbial type cell culture (MTCC), CSIR-IMTECH, Chandigarh (India).

Instruments: Fluorescence microscopic data were recorded using an inverted fluorescence microscope (Nikon Ts2-FL, Japan). Fourier Transform Infrared Spectroscopy (FTIR) (with ATR analysis facility) data were recorded using a Fourier Transform Infrared Spectrometer (Make: Agilent; Model: Cary 660 series). UV-visible spectra were collected using Shimadzu 2600 spectrophotometer. DLS data were collected using a particle size analyzer (NanoZS Zetasizer, Malvern). Thermogravimetric analysis was performed using a simultaneous thermal analyzer (Perkin Elmer, model STA 8000, USA). The above-mentioned analytical instruments are located at CIAB, Mohali. SEM images were recorded using a scanning electron microscope (JEOL, model: 6000 and JSM IT300, Japan) located at CIAB and INST, Mohali. HR-TEM analysis was performed using a TEM instrument (FEI Tecnai G2, Germany) located at NIPER, Mohali.

Preparation of Solubilized Fraction of Kraft Lignin. To prepare the ethanol-soluble fraction of the lignin, 2 g of Kraft lignin (KL) was initially added to 100 mL of absolute ethanol and then sonicated for 30 min at 25 ºC. The resulting suspension was then centrifuged at 10000 rpm for 10 minutes at 25 ºC. The supernatant containing the soluble fraction of lignin was then collected. The dried powder of the soluble lignin was obtained by removing the ethanol using a rotary evaporator. The dried powder was directly used for the synthesis of lignin nanospray (LNSR).

Optimization of Reaction Parameters for the Synthesis of Lignin Nanospray

Various reaction parameters (such as lignin concentration, reaction volume, temperature, pH, and reaction time) and components (such as the use of surfactants) were extensively optimized in order to obtain the best method for the synthesis of stable LNSR. Initially, various surfactants (10 mg/mL) e.g. CTAB, DMAB, PEG, PVP, PF-68, and Tween 80 were used.

At first, KL (10 mg/mL) in absolute ethanol was used as a stock solution. Further, 1 mL of this solution was added dropwise in the flask containing 10 mL of deionized water under sonication. The resulting reaction suspension was continued to sonicate until 10 minutes at 25 ºC. The color of the reaction mixture changed from dark brown to pale brownish color, which indicated the formation of lignin nanospray (LNSR). It was made sure that 1 mL of ethanol has been evaporated from the LNSR reaction mixture. Evaporation was done using a rotary evaporator. Synthesized LNSR was dispersed in deionized water for further characterization. To confirm the formation of LNSR, the particle size, polydispersity index (PDI), and the surface charge was determined using a Zetasizer. The effect of each surfactant on the synthesis of LNSR was investigated in terms of size, PDI, and zeta potential. The results were tabulated in Table S1. The results suggested that the synthesis of stable LNSR could be achieved without using any surfactant. Similarly, the effect of other parameters such i.e. lignin concentration (2.5–20 mg/mL, Table S2), reaction volume (2–10 mL, Table S3), reaction temperature (25–60 °C, Table S4), pH (3-11, Table S5, Figure 1), and sonication time (5-30 min, Table S6) were further optimized. The results suggested that the synthesis of LNSR could be achieved by adding 10 mg/mL of lignin-ethanolic solution in 10 mL of deionized water at pH 7 and 35 °C by sonication for 10 min (Figure S1).

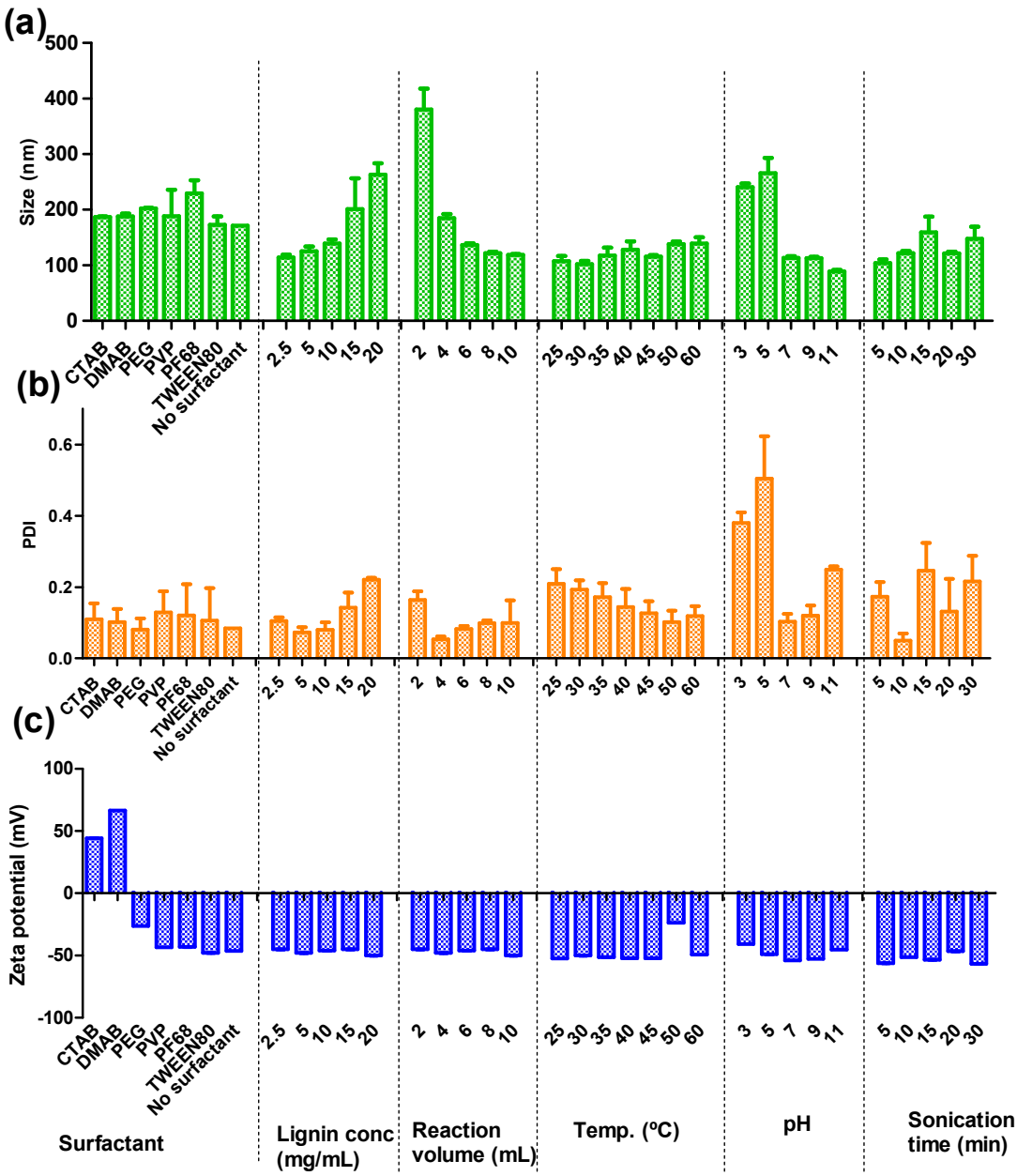

Figure S1. Graph showing the size, PDI and surface charge at different reaction parameters during optimization of the synthesis of the LNSR.

Table S1. Effect of various surfactants on the synthesis of LNSRs

Surfactant	Size (nm)	PDI	Zeta-potential (mV)

CTAB	187	0.11	44
DMAB	188	0.10	66.40
PEG	202	0.08	-26.20
PVP	188	0.12	-43.40
PF68	229	0.12	-43.10
Tween 80	172	0.10	-47.90
No Surfactant	171	0.08	-46.30

Table S2. Synthesis of LNSR at various concentrations of Kraft lignin

Lignin concentration (mg/mL)	Size (nm)	PDI	Zeta potential (mV)
2.5	114	0.10	-45.
5	125	0.07	-48.
10	140	0.08	-46.
15	201	0.14	-45.
20	263	0.22	-50.

Table S3. Synthesis of LNSR at various reaction volumes

Deionized water (mL)	Size (nm)	PDI	Zeta potential (mV)
2	380	0.16	-45
4	184	0.05	-48
6	136	0.08	-46
8	121	0.09	-45
10	118	0.10	-50

Table S4. Synthesis of LNSR at various reaction temperature

Temperature (ºC)	Size (nm)	PDI	Zeta potential (mV)
25	107	0.21	-55.40
30	102	0.19	-50
35	108	0.17	-51.20
40	128	0.14	-52.20
40	128	0.14	-52.20
45	116.	0.13	-52.20
50	138	0.10	-23.70
60	439	0.12	-49.70

Table S5. LNSR synthesis with respect to different pH

pH	Size (nm)	PDI	Zeta potential (mV)
3	138	0.27	-40.80
5	138	0.30	-48.90
7	113	0.13	-53.90
9	115	0.15	-52.80
11	85	0.18	-45.30

Table S6. LNSR synthesis with respect to different sonication time

Sonication time (min.)	Size (nm)	PDI	Zeta potential (mV)
5	101	0.17	-56.20
10	107	0.15	-51.30
15	158	0.24	-53.40
20	139	0.13	-46.50
30	144	0.21	-56.70

Characterization of the Lignin Nanosoray

UV-visible spectroscopic analysis: The UV-visible spectroscopy technique was used in order to confirm the formation of LNSR using deionized water as a solvent. The LNSR was scanned at 200-700 nm range and the data were analyzed.

Size and zeta potential analysis*:* To determine the size and polydispersity index of the LNSR, dynamic light scattering (DLS) was applied using deionized water as a solvent. To find out the charge on the surface of LNSR, the zeta potential was measured.

Electron microscopy: The morphology and size of the LNSR was analyzed using scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HR-TEM). For SEM analysis, the LNSR solution was drop casted over a silicon wafer and allowed it to vacuum dry. Then the wafer was coated with gold using sputter and analyzed under SEM. For TEM analysis, the solution of LNSR was dropped over a carbon-coated copper grid and allowed to air-dry for 15 min, then analyzed.

Fourier transform infrared (FTIR) spectroscopy: Fourier transform infrared spectroscopy-based technique named attenuated total reflectance **(**ATR) spectroscopy was used to confirm the presence of functional groups in LNSR and Kraft lignin (Figure S3 a-b). Lyophilized powdered LNSR and Kraft lignin were analyzed by ATR spectroscopy at a resolution of 4 cm^{-1} using an FTIR spectrometer. FTIR data was collected in the wavelength range of 4000-500 cm^{-1}.

Thermogravimetric analysis (TGA): The thermal stability of lignin and LNSRs was analyzed using thermogravimetric analysis with a dynamic scan from 30 to 900 °C at 20 °C/min under nitrogen atmosphere. Before being tested by TGA, the samples were dried using a lyophilizer. Thermogravimetric analysis was done for both Kraft lignin (KL) and LNSR. TGA data was collected by plotting temperature against weight loss at the range of 30°C to 900°C with heat increment rate of 20 °C/min. In the evaluated temperature range, weight decrease was observed

from 40 to 100 °C for KL, but in the case of LNSR visible weight decrease was not observed in that temperature range (Figure S3 c). The mass remained constant up to 180 °C in both compounds. After 200 °C, lowering in the curve was noticed, in both KL and LNSR, which indicated the removal of water in both species.[1] After 300 °C, a sharp decline in mass in LNSRs was observed compared to KL.[2,3]

The thermal stability of LNSRs and KL was confirmed using thermogravimetric analysis (TGA). The TGA analysis was done by plotting weight loss against temperature at the range of 30 °C to 900 °C with a heat increment rate of 20 °C min^{-1}. In temperature range from 40 to 200 ºC, a slight decrease in the weight was observed in the case of KL, which suggested the removal of bound water. In contrast, the LNSR did not show any significant weight reduction (**Figure S2 c**). After 200 °C, a significant lowering in the curve was noticed in the case of KL which suggested the removal of cellulosic and hemicellulosic impurities and further major decomposition was observed from 250 to 420 °C. .[1,2] Whilst, the LNSR showed linear and slow decomposition till 900 °C due to loss of organic component.

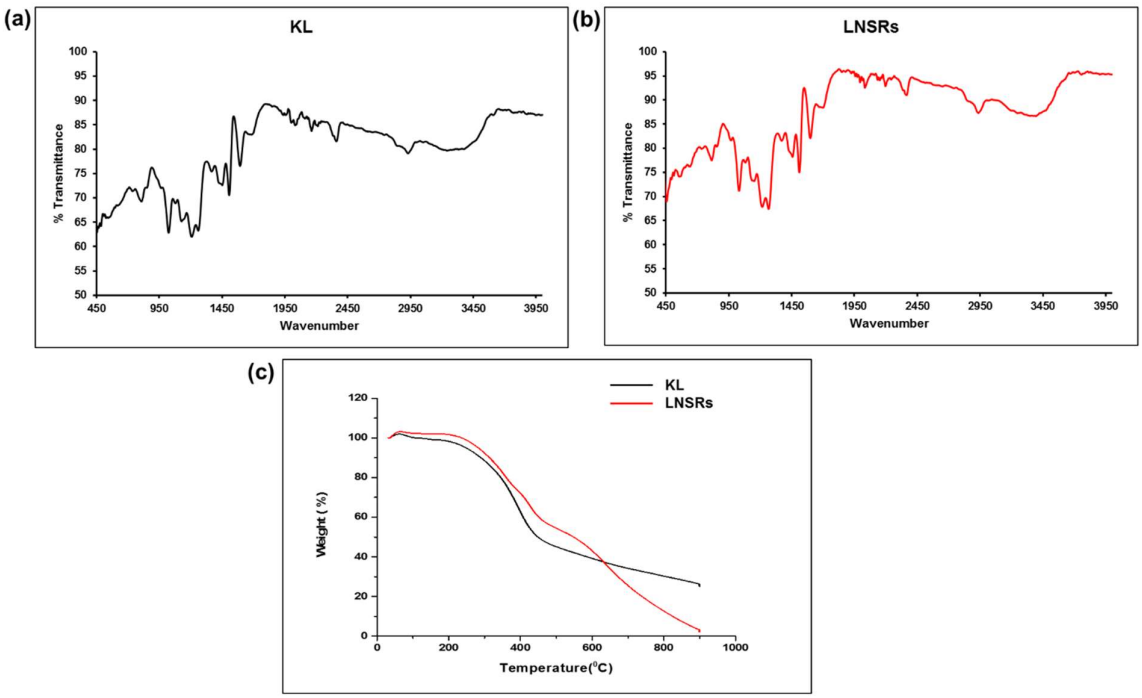

Figure S2. (a) FTIR spectra of (a) Kraft lignin, (b) LNSRs, and (c) TGA data of KL and LNSRs.

Stability Study of the Lignin Nanospray. The stability of the lignin nanospray in deionized water stored at 4 °C was checked and it was found that the lignin nanospray was stable for at least four months. During this study, the size, polydispersity index, and surface potential were monitored at every 30 day interval using DLS.

Evaluation of UV Blocking Performance of the Lignin Nanospray. In order to assess the UV-blocking capacity of LNSR, the transmittance study at different concentrations was performed using reported methods.[3,4] For this purpose, the solutions of LNSR at varying concentrations ranging from 40 to 1000 µg/mL were taken. The transmittance of each solution was recorded in the range of 200-700 nm. The percentage of transmittance of each sample in the UV-visible range was separately calculated relative to a blank solution. The transmittance behavior of the LNSR was assessed using the ultraviolet protection factor (UPF). Further, UPF was calculated for different concentrations of LNSR ranging from 40 to 1000 µg/mL in the region of both UPF_A (315-400 nm, i.e. UV-A) and UPF_B (290-320 nm, i.e. UV-B). Afterward, average UPF values were calculated for UPF_A and UPF_B using the following equations 1-3 (Table S7). The experiments were performed in triplicates.

$$UPF_A = \frac{\sum_{315}^{400}(E_\lambda \times S_\lambda \times \Delta\lambda)}{\sum_{315}^{400}(E_\lambda \times S_\lambda \times T_\lambda \times \Delta\lambda)} \quad \text{(Eq. 1)}$$

$$UPF_B = \frac{\sum_{290}^{315}(E_\lambda \times S_\lambda \times \Delta\lambda)}{\sum_{290}^{315}(E_\lambda \times S_\lambda \times T_\lambda \times \Delta\lambda)} \quad \text{(Eq. 2)}$$

$$UPF_{Average} = \frac{UPF_A + UPF_B}{2} \quad \text{(Eq. 3)}$$

E_λ = relative erythemal spectral effectiveness, S_λ = solar spectral irradiance in $W.m^{-2}.nm^{-1}$, T_λ = spectral transmittance of the LNSR, $\Delta\lambda$ = wavelength step in nm, λ = wavelength in nm.

Table S7. Ultra protection factor of lignin nanospray for UV-A and UV-B region

S. No.	Concentration (µg/mL)	UPF_A[#]	UPF_B[##]	$UPF_{Average}$	UPF
1.	40	52.72	31.50	42.11	2
2.	200	11.83	0.50	6.16	16
3.	500	3.44	0.00	1.72	58
4.	1000	0.00	0.00	1.00	100

[#]UPF_A = Ultra protection factor for UV-A region

[##]UPF_B = Ultra protection factor for UV-B region

Determination of Total Phenolic Content (TPC)

Evaluation of total phenolic content (TPC): The total phenolic content of LNSR as well as KL was estimated by following the Folin–Ciocalteu colorimetric method.[4,5] Briefly, Folin–Ciocalteu phenol reagent (200 µL) was added into separate vials containing the solutions of lignin and LNSR (50 µg/mL each) at room temperature. After 1 min, the aqueous solution of Na_2CO_3 (2% w/v, 100 µL) was added to the mixture with vigorous shaking and the resulting mixture was incubated for 30 minutes in dark at 25 ºC. The final reaction volume was made up to 1 mL. The absorbance of the resulting solution was recorded at 760 nm using a UV-vis spectrophotometer. Gallic acid was used as a standard to express the total phenolic content into the sample as its equivalent concentration. The standard calibration curve of gallic acid was plotted using its different concentrations in the range of 0-70 µL/mL. Further, equivalent gallic acid content in the test samples was determined using the standard linear equation (A = 0.0246c, R^2 = 0.9932, figure S3, Table S8). The experiments were conducted in triplicates.

Table S8. Standard calibration table of different concentrations of gallic acid (10 to 50 µg/mL)

Gallic Acid (µg/mL)	FC(µL)	Na_2CO_3(µL)	Final volume (µL)	Average (O.D.) n=3
0	200	100	1000	0.00
10	200	100	1000	0.26
20	200	100	1000	0.51
30	200	100	1000	0.66
40	200	100	1000	0.98
50	200	100	1000	1.27

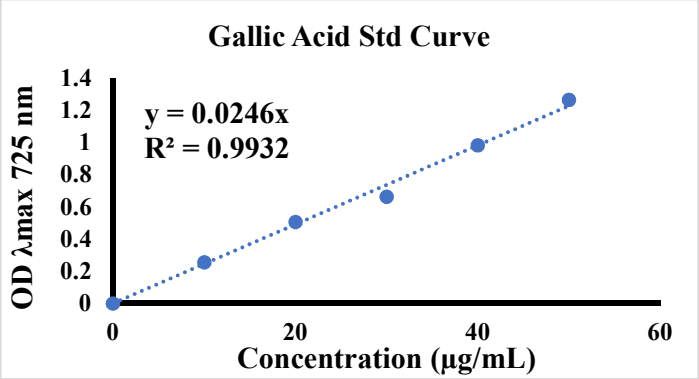

Figure S3. Standard calibration curve of the total phenolic content of gallic acid

Table S9. Total phenolic content of the LNSRs and KL

Sample (µg/mL)	FC (µL)	Na_2CO_3 (µL)	Final volume (µL)	Average OD @ 760 nm	Total Phenolic Content (GAE) (µg/mL)	Percentage of TPC (%)
LNSR (50)	200	100	1000	0.44	17.81	35.63
KL (50)	200	100	1000	0.34	13.61	27.23

Determination of Total Flavonoid Content (TFC)

Evaluation of total flavonoid content (TFC): To determine the total flavonoid content present in LNSR and KL, aluminum chloride ($AlCl_3$) colorimetric method was used.[6] Briefly, 2% (w/v) $AlCl_3$ (400 µL) was mixed with each sample of LNSR and Kraft lignin (50, 100, and 200 µg/mL) at room temperature. Deionized water was then added to the reaction mixture to make

the final reaction volume up to 1 mL. It was then allowed to stand for 1 hour at room temperature in dark. The absorbance of the resulting solution was recorded at 510 nm using a UV-Vis spectrophotometer. The standard curve using quercetin dihydrate was also prepared at different concentrations (0, 100, 200, 300, 400 µl) prior to the study of the test samples, the final reaction volume was made up to 1 mL. The standard linear equation (A = 0.0031c, R^2 = 0.98, Figure S4, Table S10) derived from the standard curve was used to determine quercetin dihydrate equivalent (QDE) concentration in test samples. The QDE concentration was referred to as the flavonoid content in the test samples. The experiments were conducted in triplicates.

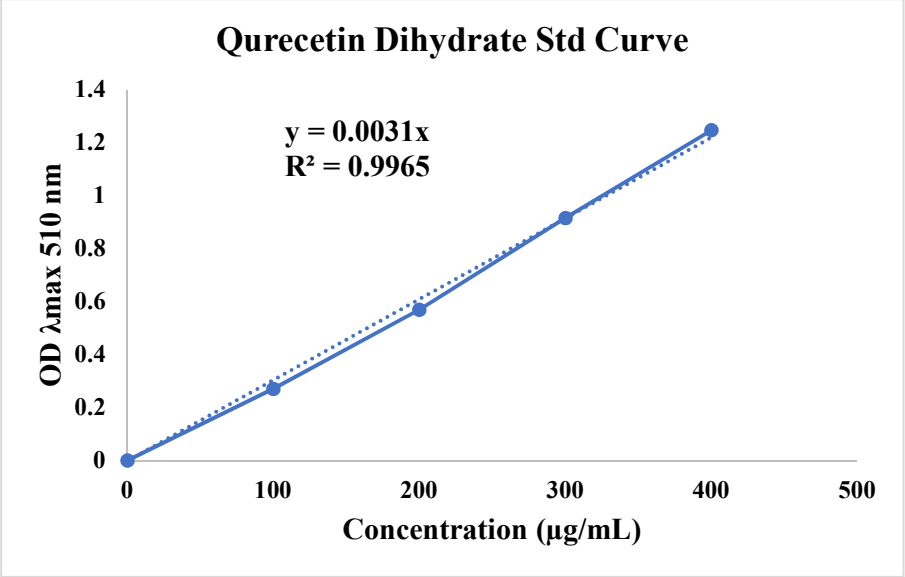

Figure S4. Standard calibration curve of total flavonoid content of quercetin dehydrate.

Table S10. Total flavonoid content of the LNSRs and KL

Sample (µg/mL)	$AlCl_3$ 2% w/v (µL)	Average OD @ λ_{max} 510 (n=3)	Total flavonoid content (QDE) (µg/mL)	Percentage of TFC (%)
LNSRs (50)	400	0.07	23.76	47.52
LNSRs (100)	400	0.13	42.36	42.36
LNSRs (200)	400	0.22	73.11	36.55
KL (50)	400	0.06	17.31	34.62

KL (100)	400	0.10	31.82	31.82
KL (200)	400	0.13	40.43	20.21

Determination of Antioxidant Property of LNSR via ABTS Assay

Antioxidant Activity of Lignin Nanospray. The antioxidant potential of the LNSR and Kraft lignin was determined to evaluate their capacity to scavenge ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical cations. The antioxidant potential of LNSR was determined using the ABTS radical scavenging method while ascorbic acid was used as a standard (Figure S5, Table S11).[3,5] The standard graph of ascorbic acid at varying concentrations of 0-3.5 μg/mL was plotted and the linear equation was determined for further quantification. Simulataneously, ABTS radical cations were produced by reacting 7 mM ABTS with 5 mM potassium persulfate ($K_2S_2O_8$) in deionized water by incubating the reaction mixture in dark at room temperature for 12 h. Then, in 100 μL of ABTS radical cation solution, different concentrations (1-20 μg/mL, the final volume of the reaction mixture was set to 200 μL) of test samples were added. The resulting solutions were incubated for 5 min in dark and then the absorbance of each sample was recorded at 734 nm (Table S12). The percentage of radical scavenging by the test sample was calculated with respect to the blank solution containing no scavenger. The sample concentration providing 50% of radical scavenging activity (EC_{50}) was calculated from the graph of ABTS radical cation scavenging. Percent scavenging of ABTS radical was determined using the following equation 4.

$$percentage\ scavenging\ (\%) = \frac{[(Abs\ \text{control} - \text{Abs sample})]}{Abs\ control} * 100 \qquad \text{(Eq. 4)}$$

The reactions were conducted in triplicates.

Table S11. Standard ascorbic acid (AA) inhibiting ABTS radical ions

Concentration of AA (μg/mL)	Average OD @ λmax 734 (n=3)
0	1.03

0.5	0.93
1	0.86
1.5	0.76
2	0.67
2.5	0.55
3	0.47
3.5	0.37

Table S12. Antioxidant activity of LNSR with respect to different concentrations

Concentration of Sample (µg/mL)	**Average OD of LNSR @ λmax 734 (n=3)**	**LNSRs average Equiv conc. of AA**	**Scavenging effect using ABTS assay (%)**
0	1.09	0.26 ± 0.030	0
1	0.88	0.83 ± 0.080	19.26
2.5	0.71	1.77 ± 0.060	34.86
5	0.53	2.65 ± 0.070	51.37
10	0.25	4.15 ± 0.070	77.06
15	0.13	4.77 ± 0.030	88.07
20	0.10	4.96 ± 0.060	90.820

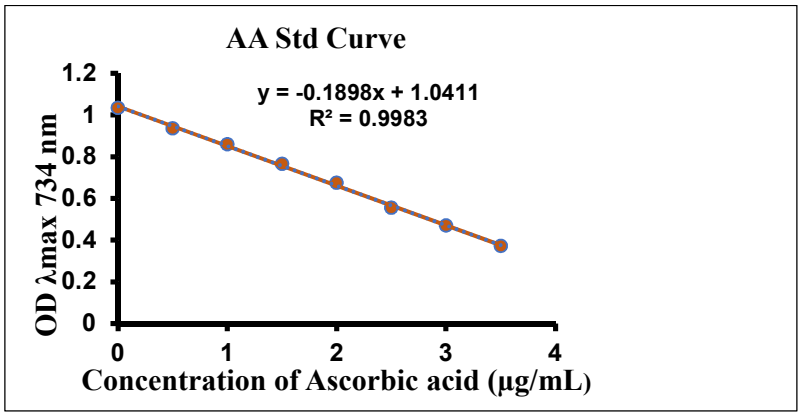

Figure S5. Standard calibration curve of ascorbic acid.

Calculation:

y = - 0.1898x + 1.0411

OD of ABTS @ 2.5 µg of LNSR = 0.7123 (y)

Then,

=> 0.7123 = - 0.1898x + 1.0411

=> 0.7123 - 1.0411 = - 0.1898x

=> - 0.3288 = - 0.1898x

=> x = 0.3288 / 0.1898

=> x = 1.73 µg of AA

It means 2.5 µg of LNSR having equivalent activity of 1.73 µg of AA

Thus, the IC_{50} value of LNSR can be calculated as following:

=> 1.73 µg AA ≈ 2.5 µg of LNSR

IC_{50} value of LNSR

=> 2.76 µg AA = 2.5 / 1.73 * 2.76

=> 2.76 µg AA ≈ 3.99 µg

Hence, 3.99 µg IC_{50} value of LNSR

Determination of Photoluminescence Property of LNSR

Table S13: Fluorescence quantum yields of developed LNSR and standard

Material	**Integrated intensity (n=3)**	**Absorbance excitation wavelength (n=3)**	**Fluorescence quantum yield (Φ_f)**
Rh 6G Standard [a]	69228	0.001	0.90
LNSR [a]	2316.1	0.080	0.03

[a] λex = 480 nm

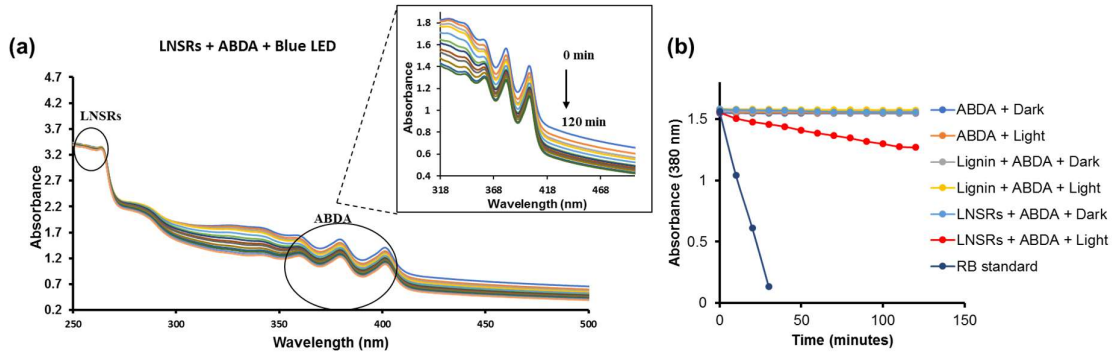

Figure S6. (a) Graph showing absorption spectra of ABDA solution treated with LNSRs and inset graph depicts LNSRs and ABDA mixture irradiated with a blue LED at different time intervals. (b) Graph showing absorption spectra of ABDA solution treated with LNSRs and lignin in absence with blue LED at different time intervals.

Evaluation of Singlet Oxygen Quantum Yield of LNSRs

Table 14: Singlet oxygen quantum yield (Φ_Δ) of different probes[#]

S. No.	Probe	SOQY (Φ_Δ)
1.	ABDA + Dark	0.003
2.	ABDA + Light	0.03
3.	Lignin + Dark	0.05
4.	Lignin + Light	0.11
5.	Lignin nanospray + Dark	0.13
6.	Lignin nanospray + Light	0.35

[#]The experiments were performed in triplicates

Elucidation of Mechanism of Photoinduced Antimicrobial Action of the Lignin Nanospray

***Evaluation of Nucleic Acid Leakage from LNSR Treated Bacteria*:** To evaluate nucleic acid leakage from LNSR treated cells a reported method was followed.[5] For this purpose, bacterial cells (OD_{600} 0.6, suspended in PBS buffer) were incubated individually with LNSR and KL at

37 °C for 60 min in dark. The cell suspensions containing LNSR and KL were further irradiated with a blue LED (12 W) for 0, 30, and 60 minutes, respectively. Afterward, the treated cells were centrifuged at 8000 rpm for 5 min at 4 ºC. The resulting supernatants were filtered using 0.22 μm syringe filters. The obtained filtrates were analyzed at 260 nm using a UV−visible spectrophotometer to detect released DNA from the cells.

Measurement of Surface Potential of LNSR Treated Cells: To determine the interaction in the surface charge of the LNSR and KL with cells a reported method was used.[5] After treatment of cells with the probes in the presence of blue LED (12 W) at different time intervals (at dark, after 30 and 60 minutes), the zeta potential was measured for each of the reaction mixtures using a particle size analyzer.

Determination of Morphological Changes in the Lignin Nanospray Treated Bacterial Cells. The morphological alterations in the cell structure after the treatment were determined using SEM analysis. In this study, bacterial cells treated with LNSRs were centrifuged at 8000 rpm for 5 minutes. The pellets were washed and resuspended in PBS. The cells were fixed on a silicon wafer using 5% glutaraldehyde and then vacuum-dried.[5] Then the fixed and dried cells were gold-sputtered and analyzed under a scanning electron microscope.

Determination of the IC_{50} Values of Various Samples Against Microorganisms

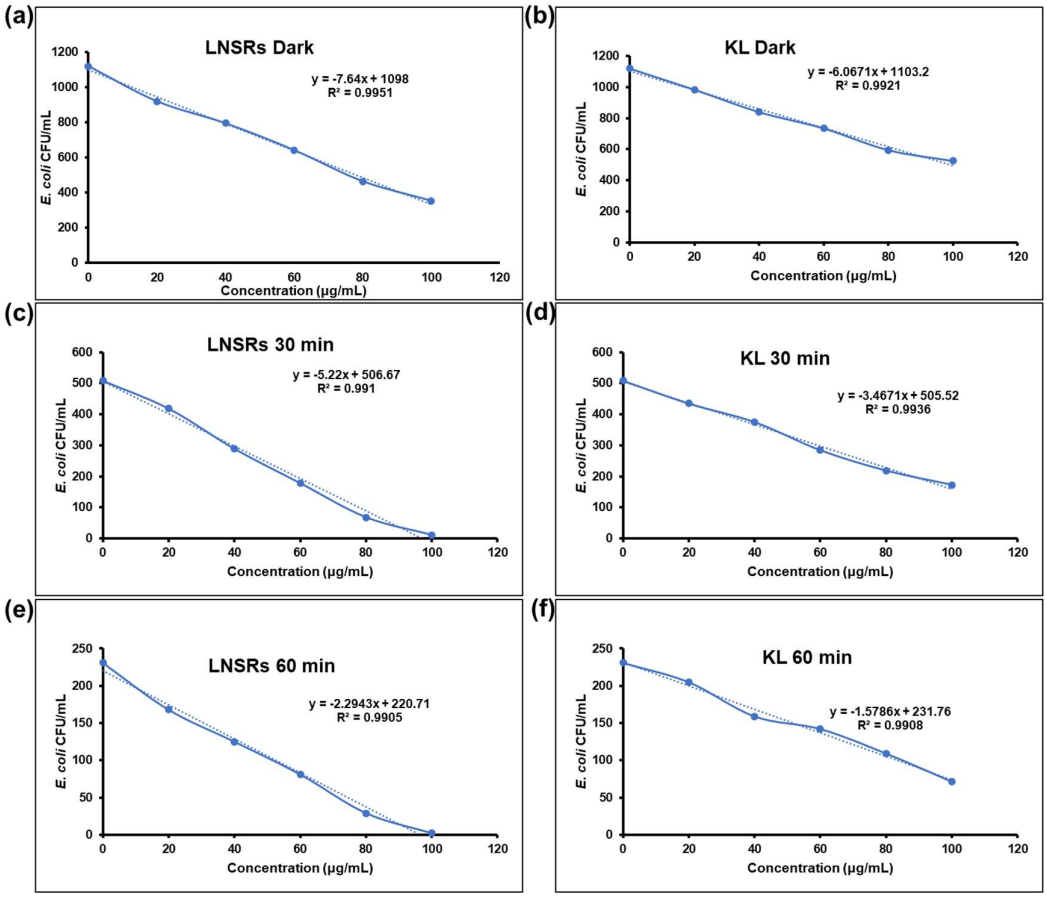

Figure S7. Graphs to determine the IC_{50} values of various samples against *E. coli*: (a) LNSRs at dark (b) KL at dark, (c) LNSRs after 30 min light exposure, (d) KL after 30 min light exposure, (e) LNSRs after 60 min light exposure and (f) KL after 60 min light exposure.

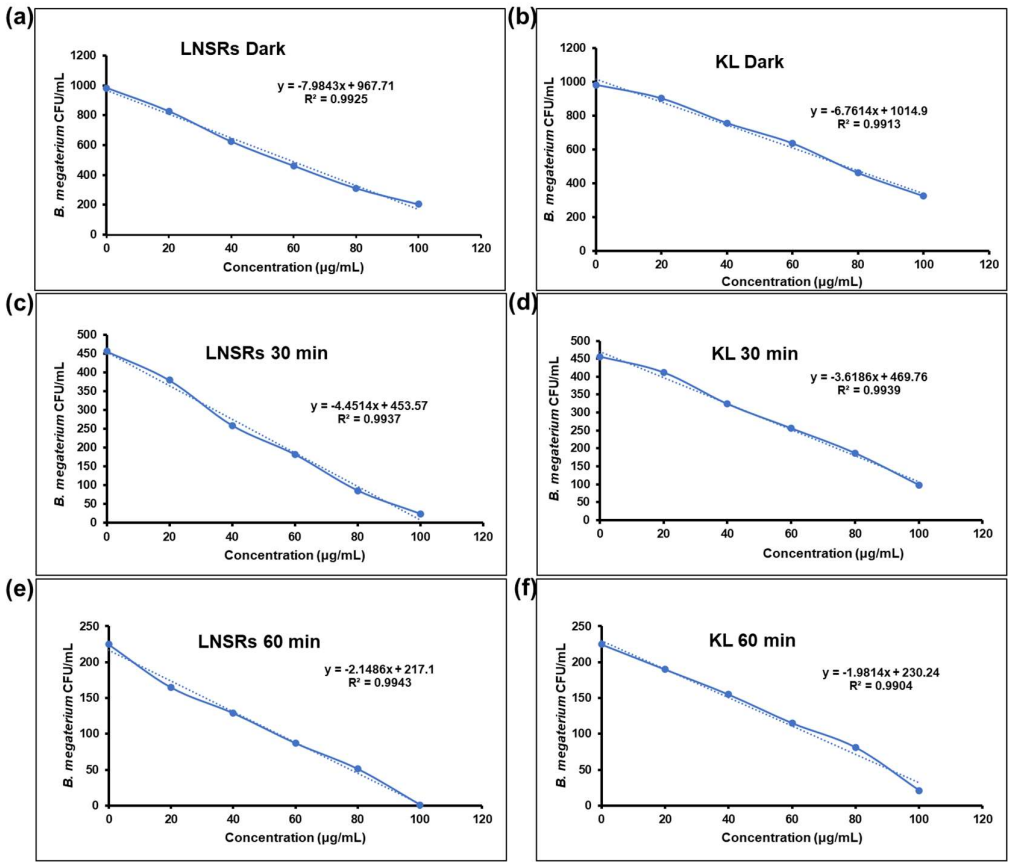

Figure S8. Graphs to determine the IC50 values of various samples against *B. megaterium:* (a) LNSRs at dark (b) KL at dark, (c) LNSRs after 30 min light exposure, (d) KL after 30 min light exposure, (e) LNSRs after 60 min light exposure and (f) KL after 60 min light exposure.

References

1 S. Chu, A. V. Subrahmanyam and G. W. Huber, *Green Chem.*, 2013, **15**, 125–136.

2 J. Y. Kim, E. J. Shin, I. Y. Eom, K. Won, Y. H. Kim, D. Choi, I. G. Choi and J. W. Choi, *Bioresour. Technol.*, 2011, **102**, 9020–9025.

3 R. Kaur, N. S. Thakur, S. Chandna and J. Bhaumik, *J. Mater. Chem. B*, 2020, **8**, 260–269.

4 Australian/New Zealand Standard, AS/NZS 4399:2017: sun protective clothing – evaluation and classification - ResearchOnline@JCU. (n.d.). Retrieved August 11, 2020, from https://researchonline.jcu.edu.au/52549/an/New Zealand Standard, AS/NZS 4399:2, https://researchonline.jcu.edu.au/52549/, (accessed 11 August 2020).

5 S. Chandna, N. S. Thakur, Y. N. Reddy, R. Kaur and J. Bhaumik, *ACS Biomater. Sci. Eng.*, 2019, **5**, 3212–3227.