Supporting Information for

Unlocking the potential of agri-biomass based lignin derived zinc oxide nanocomposites as promising UV protectant-cum-antimicrobial agents

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Table of Contents

S1. Instruments

FTIR (with ATR analysis facility) data were recorded using a Fourier Transform Infrared Spectrometer (Make: Agilent; Model: Cary 660 series) at CIAB. UV-visible spectra were collected using Shimadzu 2600 spectrophotometer at CIAB, Mohali. DLS data were collected using a particle size analyzer (NanoZS Zetasizer, Malvern) at CIAB and NABI, Mohali. HR-TEM and EDX analysis were performed using a TEM instrument (FEI Tecnai G2, Germany) at NIPER, Mohali. SEM images were recorded using a scanning electron microscope (Model: JSM IT300) at INST, Mohali. XRD data was collected using a Rigaku Ultima IV X-ray diffractometer, at IISER, Mohali.

S2. Experimental Section

Alkali lignin from commercial source has pH 10.5, zeta size 2.19 μm and zeta potential l-91 mV, whereas kraft lignin has pH approx. 7, zeta size 1.71 μm and zeta potential -22.8 mV.

S2.1. Preparation of Lignin Stock Solution

S2.1.1. Commercial Alkali Lignin: Lignin stock solution (5 mg/mL) was prepared by dissolving lignin powder directly in deionized water. The solution was stirred using a magnetic stirrer for 30 min at room temperature for homogenous mixing.

S2.1.2. Kraft Lignin: Kraft lignin (obtained from agricultural biomass) was crushed using a pestle mortar to obtain a fine powder. The powder (4 mg) was then dissolved in deionized water (1 mL). In the above solution 2M NaOH was added dropwise till the homogenous solution was obtained. The resulting solution was then stirred at 60 °C for 2 h.

S2.2. Optimization of Physicochemical Parameters to Synthesize ZnOAL and ZnOKL Nanocomposites

Various reaction conditions, such as Zn salt concentration, lignin concentration, pH, temperature, stirring/ultrasonication, drying temperature and time duration were optimized to obtain ZnONCs. All the experiments for optimization of the parameters were performed in triplicates. Lignin stock solution concentration was varied from 0.5 mL to 16 mg/mL. Concentration of zinc salts were varied from 0.01 to 0.1 M. The reaction temperature was optimized in the range of 25 to 125 ºC. The pH of the reaction mixture was optimized in the range of 4 to 12. The ideal reaction time was determined by characterizing the reaction mixture at different time intervals (2, 4, 6, 8, 16, 24, and 48 h). Moreover, the reaction efficiency was monitored on a magnetic stirrer and a bath sonicator. Synthesized nanocomposites were

initially characterized without drying and then characterized after drying in the oven at a varying temperature between 80 to 110 ºC for different time duration (4 h to 24 h).

S2.3. Characterization of Nanocomposites

S2.3.1. UV-Visible Spectroscopy: For UV–visible spectroscopy, a sample solution of ZnOAL and ZnOKL nanocomposites from each of the reaction mixtures was diluted with equal volume of ethanol. The absorption spectra were then recorded (200–800 nm) using a UV-Vis spectrophotometer (Figure S1, Table S1).

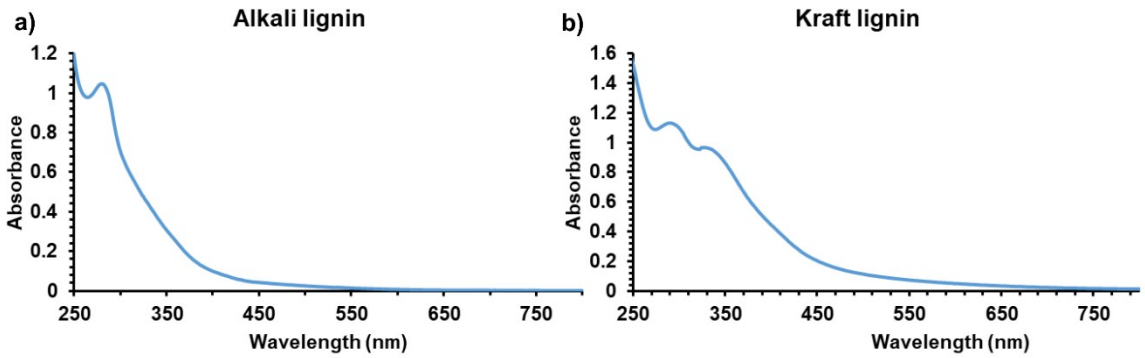

Figure S1. UV-Vis spectra of a) alkali lignin and b) kraft lignin.

S2.3.2. Particle Size Analysis: The ZnOAL and ZnOKL nanocomposites were suspended in ethanol and sonicated for 10 min prior to the analysis. Particle size analysis of each of the nanocomposites suspension was performed using a Zetasizer (Table S1, Figure S2 a-d).

Table S1. Characterization data of the nanocomposites.

Nanocomposite type	**UV-Vis Absorption maxima (nm)**	**Zeta Potential (mV)**	**Size (nm)**
ZnOAL nanocomposite	300	-38.3	372
ZnOKL nanocomposite	350	-20	196
Kraft lignin	289	-22	1713
Alkali lignin	280	-90	2191

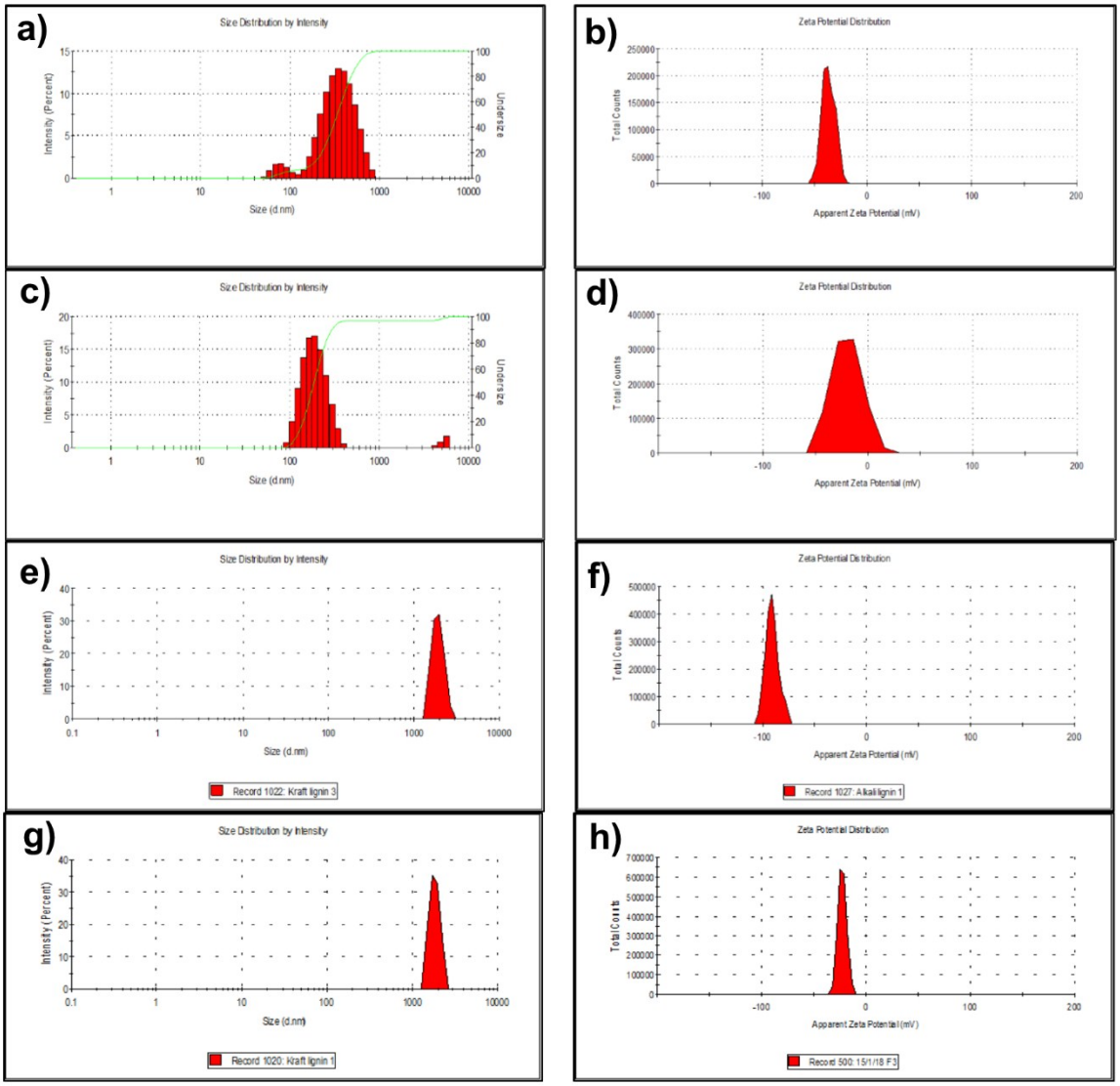

Figure S2. (a) The size distribution and (b) zeta potential graphs of ZnOAL nanocomposite; (c) The size distribution and (d) zeta potential graphs of ZnOKL nanocomposite; (e) The size distribution and (f) zeta potential graphs of alkali lignin; (g) The size distribution and (h) zeta potential graphs of kraft lignin.

S2.3.3. Scanning Electron Microscopy (SEM): The shape and size of the nanocomposites were further analysed using scanning electron microscopy (SEM), dried nanocomposite sample was placed on a carbon tape, coated with gold and observed under the SEM.

S2.3.4. High Resolution-Transmission Electron Microscopy (HR-TEM) and Electron Dispersive X-Ray (EDX) Spectroscopy: Size, shape, and composition of nanocomposites were analysed using HR-TEM and EDX analysis. Nanocomposites were suspended in ethanol and sonicated for 20 min prior to placing the sample on a copper grid. The sample was the deposited and dried on top of the grid followed by analysis under TEM (Figure 2 b&f, S3 a & b).

S2.3.5. Powder X-Ray Diffraction (XRD) Analysis: The ZnONC powder samples were tightly packed into the sample holders. X-ray diffraction patterns of the ZnONCs samples were obtained by using a fully automatic high-resolution powder X-ray diffractometer system with 2θ = 2-80° (Figures 2 d and h).

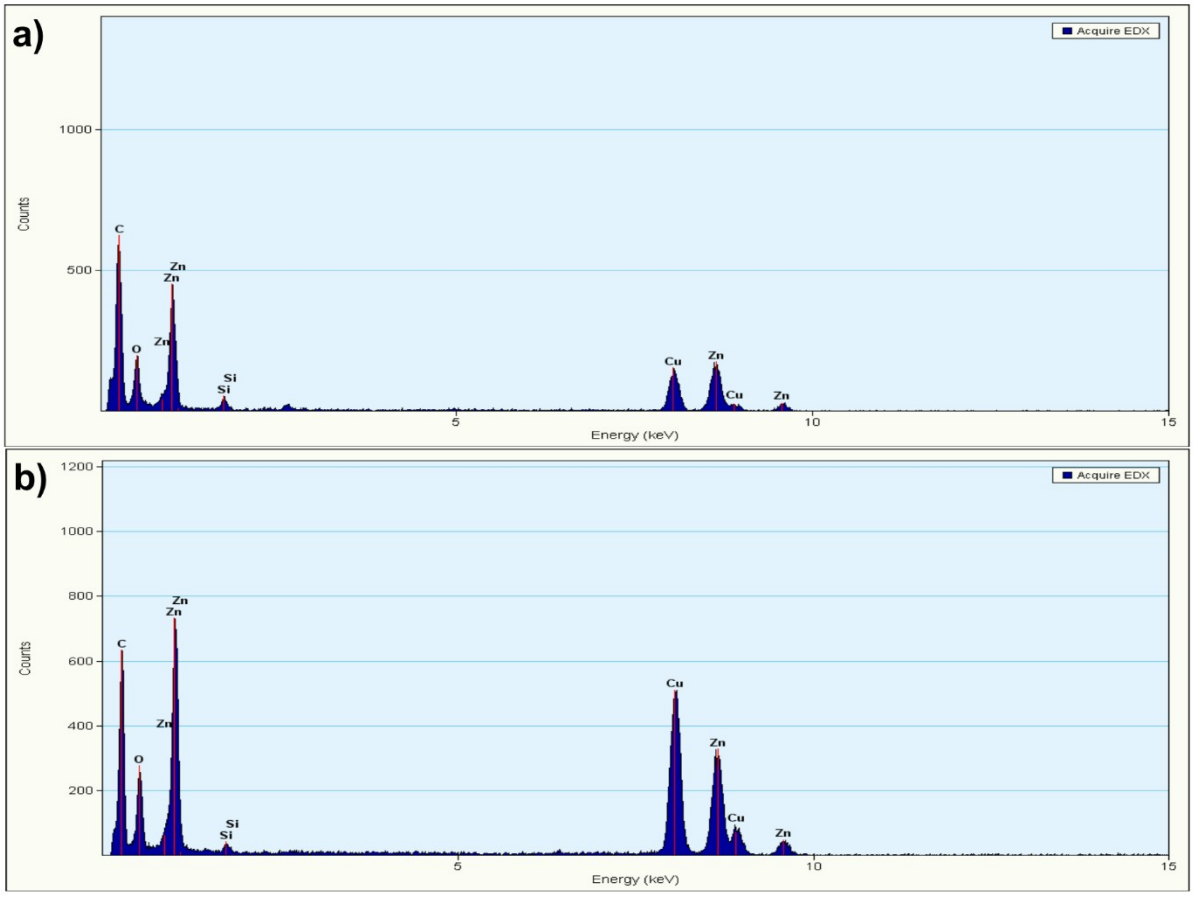

Figure S3. EDX analysis of a) ZnOAL and b) ZnOKL nanocomposites.

S2.3.6. Fourier Transform Infrared (FTIR) Spectroscopy: The presence of functional groups on surface of ZnONCs were analyzed by FTIR spectroscopy analysis. Each sample (5 mg) in dried form was placed on the sample holder and FTIR spectra was recorded on an FTIR spectrometer using self-supported pellet technique (Figure S4 a-c; Figure S5 a & b).

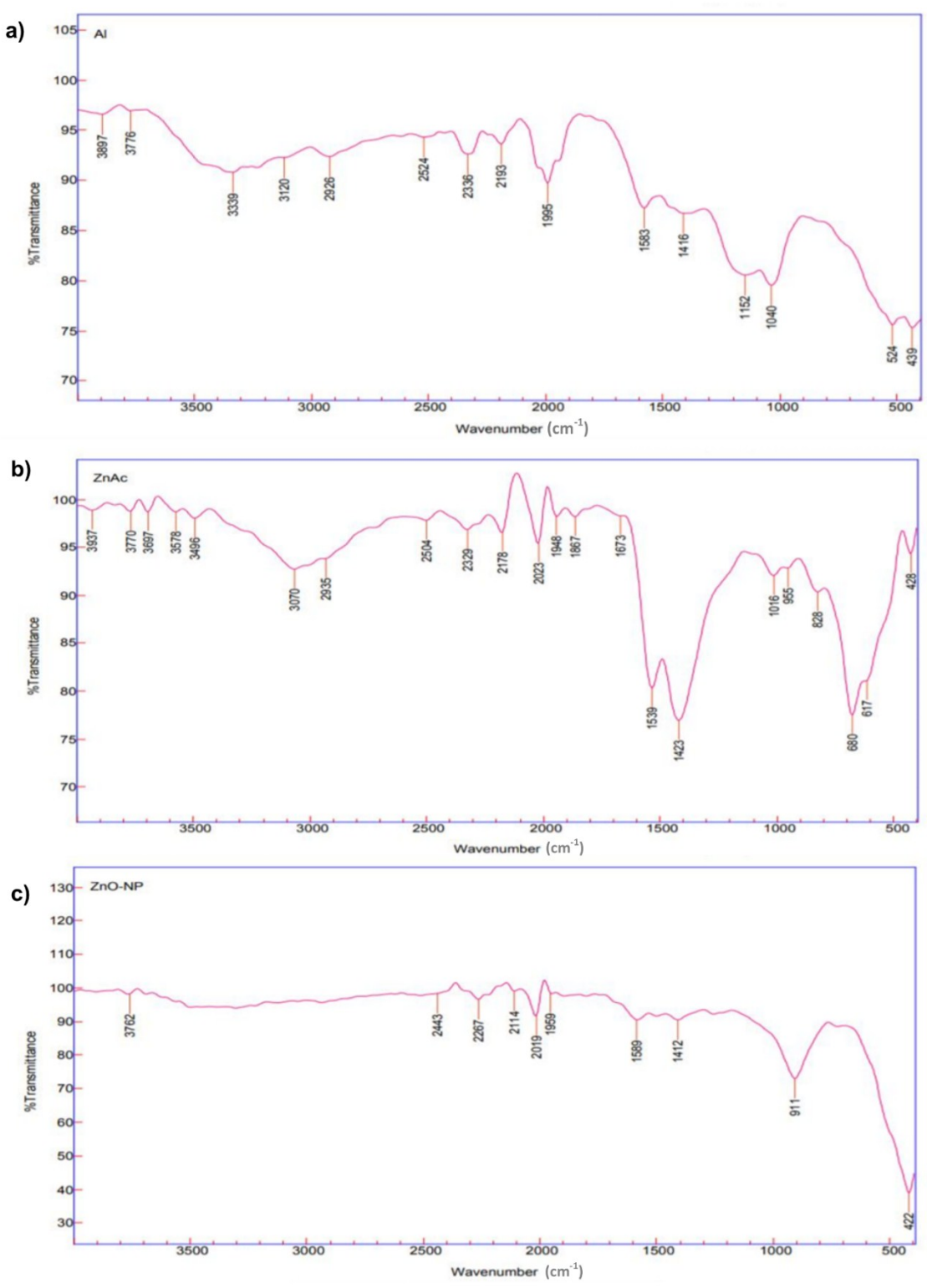

Figure S4. FTIR spectra of (a) alkali lignin, (b) zinc acetate salt and (c) ZnOAL nanocomposite.

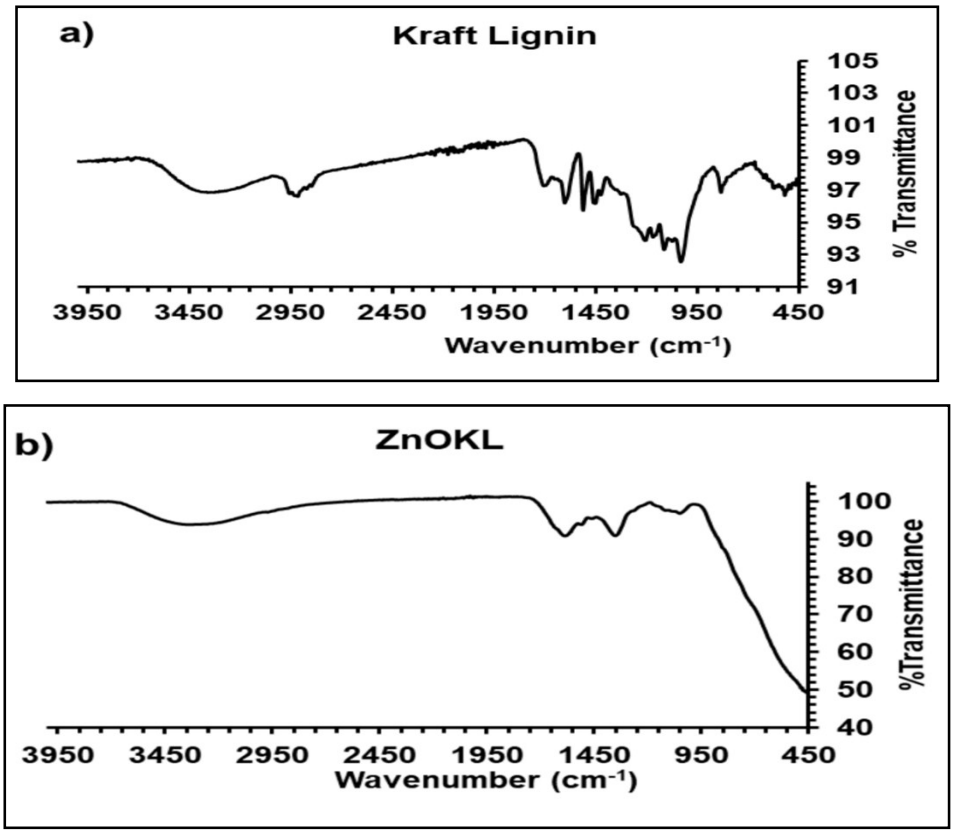

Figure S5. FTIR spectra of a) kraft lignin and b) ZnOKL nanocomposite.

S.2.4. Determination of conjugation ability of the ZnO nanocomposites

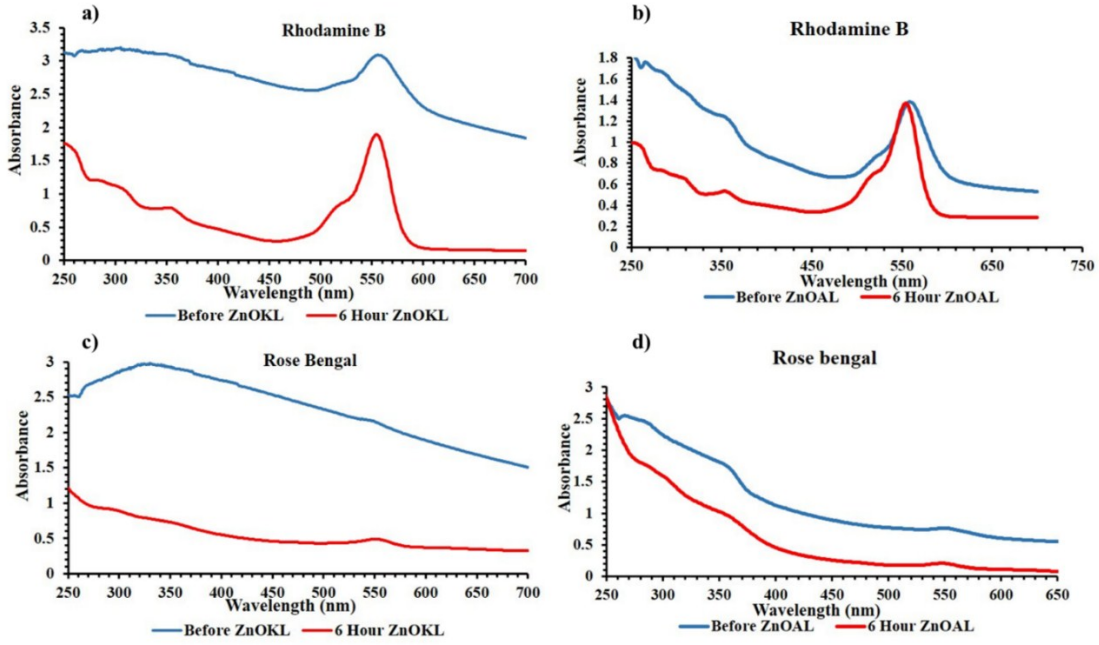

Figure S6. UV-visible spectra of nanoconjugates: a) Rhodamine B-ZnOKL, b) Rhodamine-ZnOAL, c) Rose Bengal-ZnOKL and d) Rose Bengal-ZnOAL. Blue: UV-Vis spectra of conjugate before NaOH treatment; Red spectra: UV-vis spectra of the nanoconjugate after NaOH treatment.

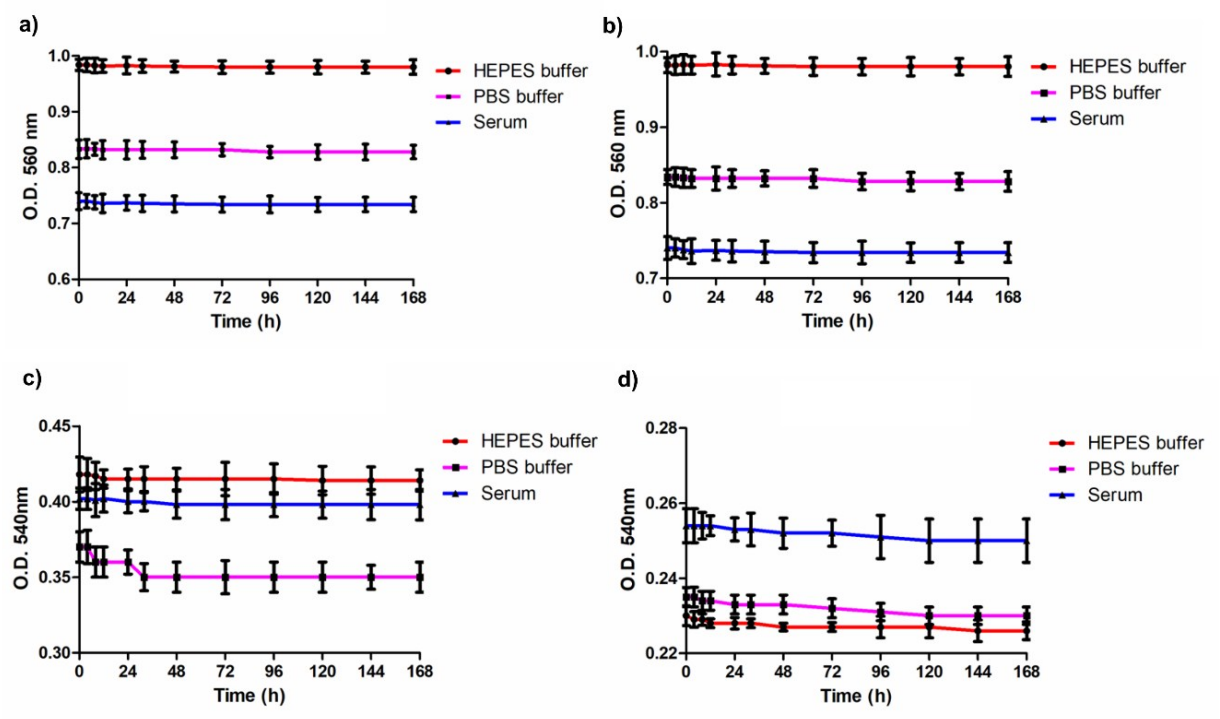

Figure S7. The absorbance of a) rose bengal-ZnOAL nanoconjugates, b) rose bengal-ZnOKL nanoconjugates, c) rhodamine B-ZnOAL nanoconjugates and d) rhodamine B-ZnOKL nanoconjugates against time at various time intervals (upto 7 days) under physiological conditions.

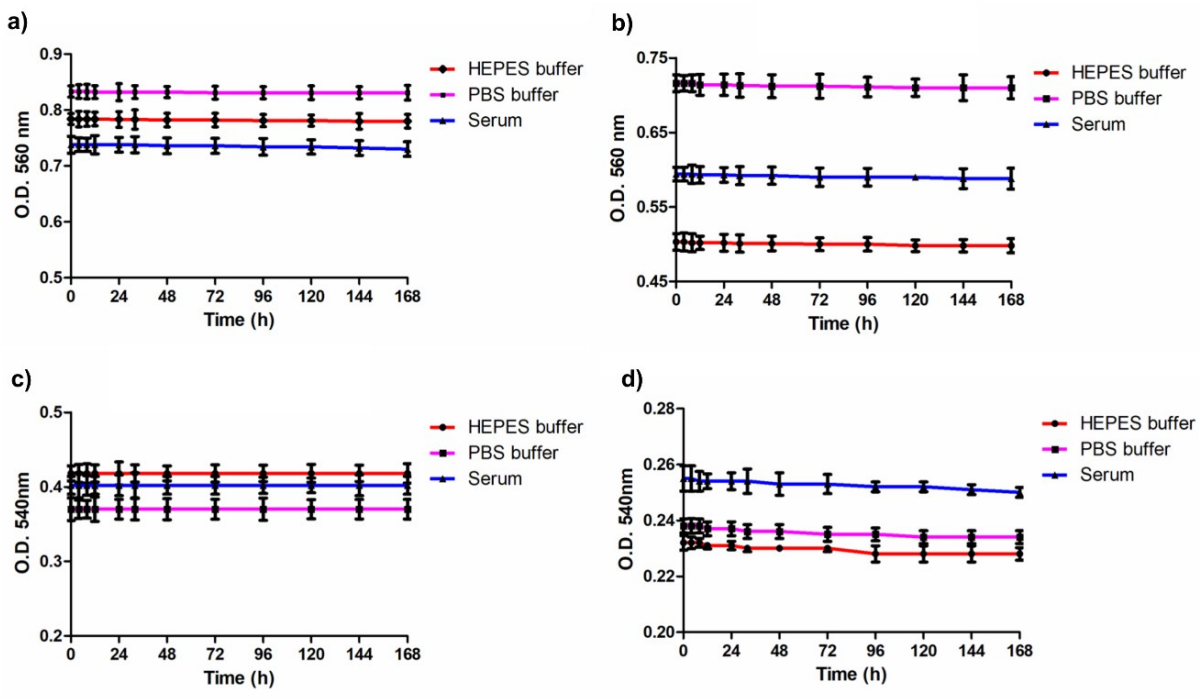

Figure S8. The absorbance of a) rose bengal-ZnOAL nanoconjugates, b) rose bengal -ZnOKL nanoconjugates, c) rhodhamine B-ZnOAL nanoconjugates and d) rhodhamine B-ZnOKL nanoconjugates against time at various time intervals (upto 7 days) under storage conditions.

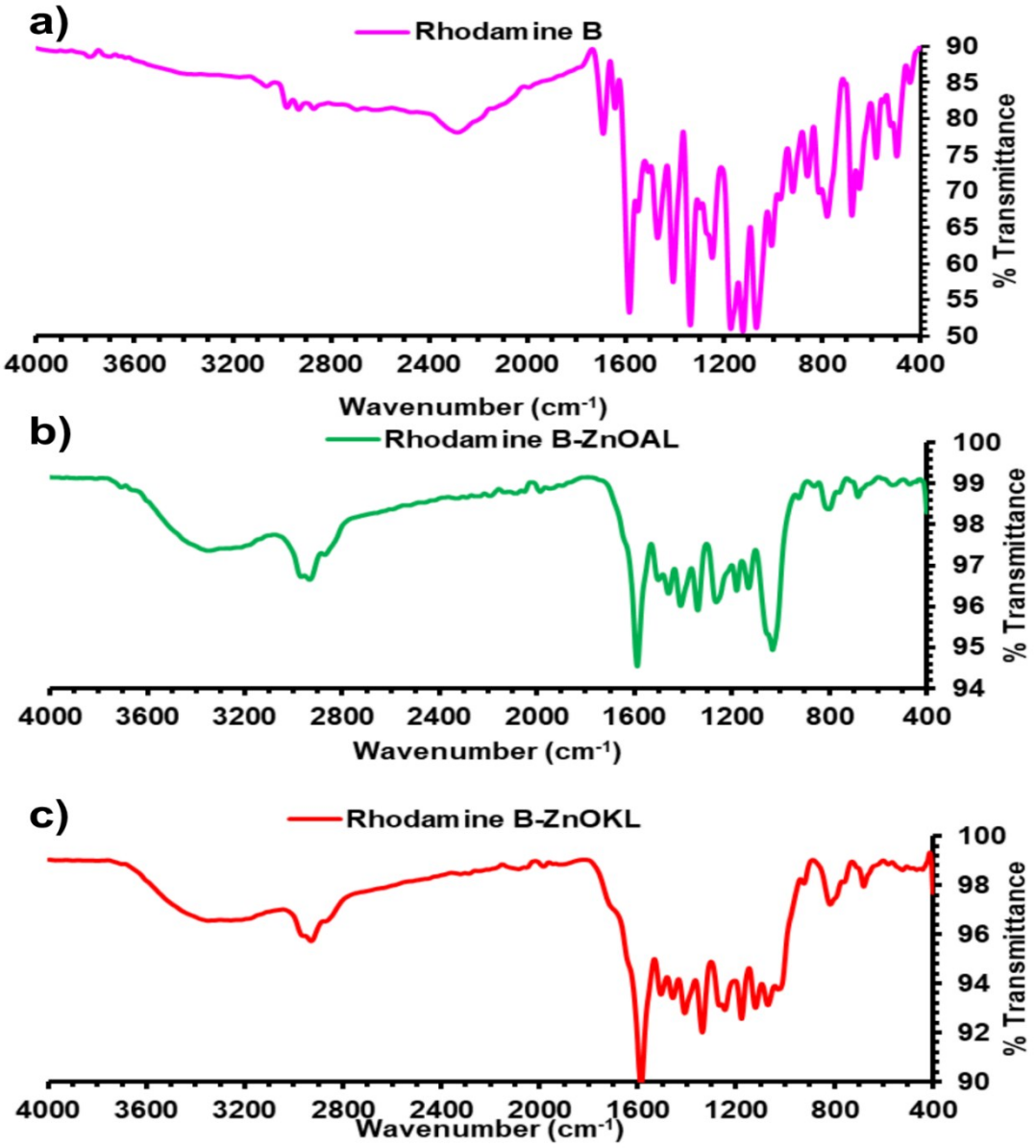

Figure S9. FTIR spectra of a) Rhodamine B, b) Rhodamine B-ZnOAL nanoconjugate and c) Rhodamine B-ZnOKL nanoconjugate.

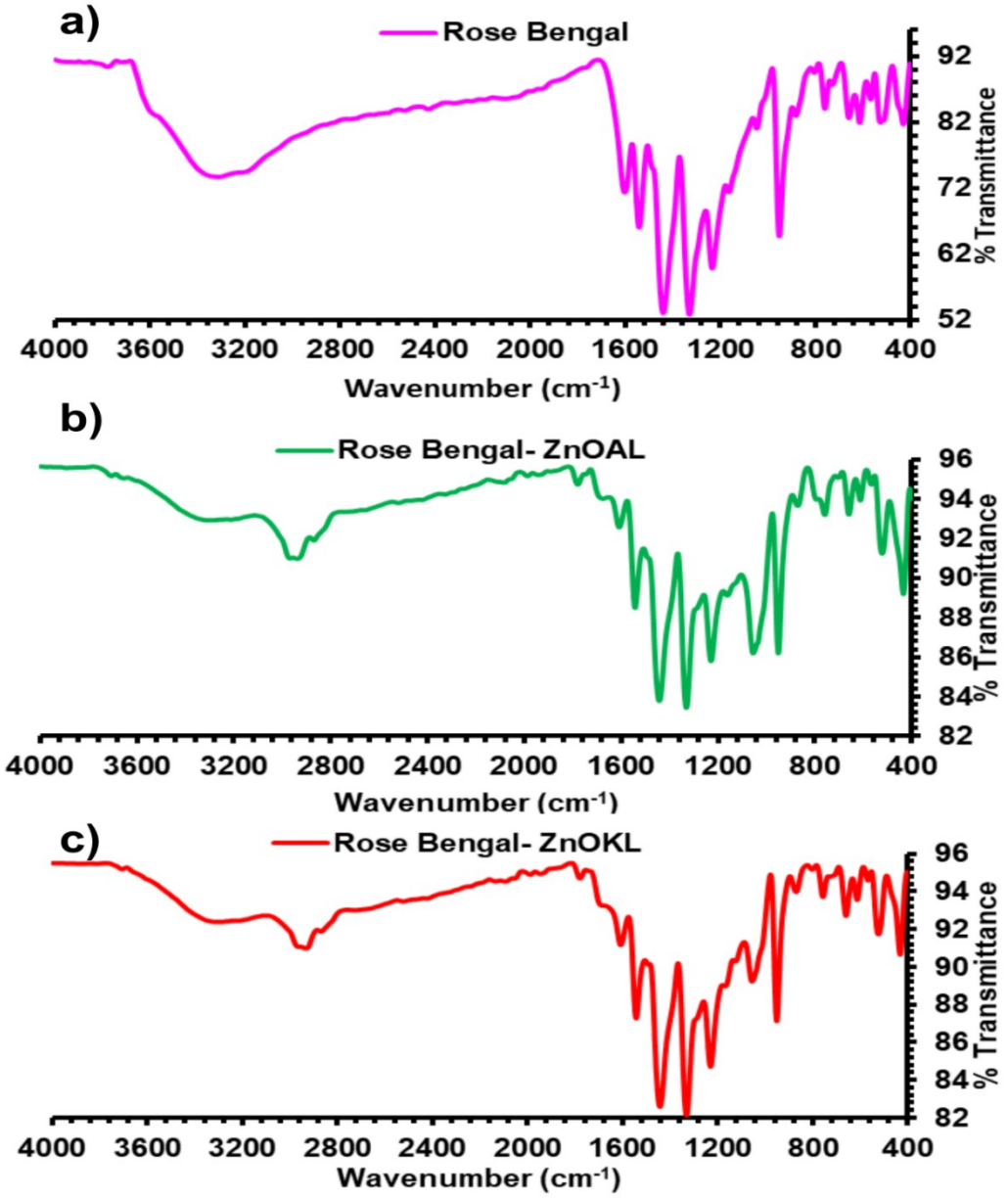

Figure S10. FTIR spectra of a) Rose bengal, b) Rose bengal-ZnOAL nanocomposite (nanoconjugate) and c) Rose bengal-ZnOKL nanocomposite (nanoconjugate).

S.2.5. Determination of lignin loading on nanocomposites

The amount of lignin loaded on the ZnO nanocomposites is calculated using equation 1.

$$\text{Lignin loading (\%)} = (Abs_{initial} - Abs_{supernatant})/Abs_{initial} * 100 \quad \text{(Eq. 1)}$$

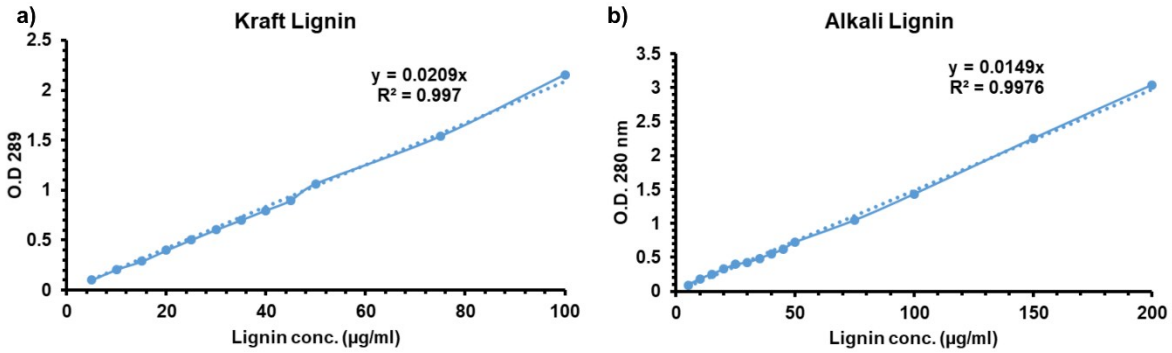

Figure S11. Standard calibration curve of a) kraft lignin and b) alkali lignin.

S.2.6. Determination of antioxidant potential of ZnO nanocomposites

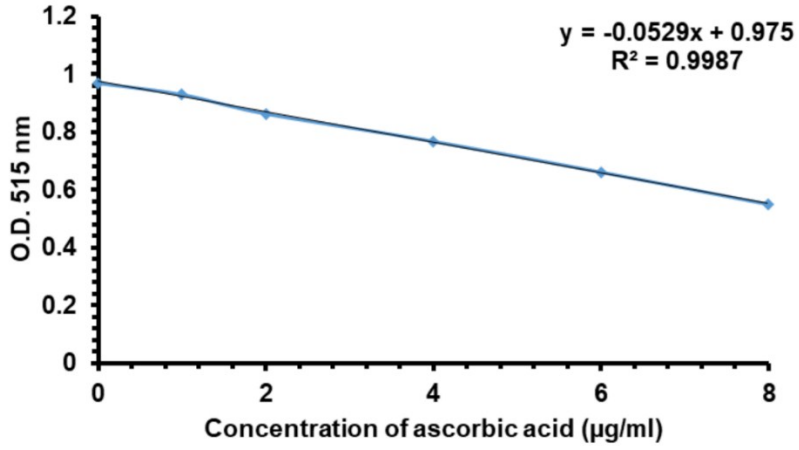

Figure S12. Standard calibration curve of ascorbic acid.